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TABLE OF CONTENTS

Vapor action of certain fungicidal materials prepared for dusting cotton seed ..... S. G. LEHMAN 431

A destructive virus disease of sour cherry.
G. W. KEITT AND C. N. CLAYTON 449

Pathogenicity studies with Fusaria isolated from tobacco, sweet potato, and cotton ..... T. E. SMITH AND K. J. SHAW 469

A browning reaction to stem rust in wheat.
HELEN HART AND J. LEWIS ALLISON 484

Physical characteristics of Bordeaux mixture in relation to its qualities ..... E. E. WILSON 497

Tests of eradicant sprays for use against Sclerotinia laxa and Coryneum beijerinckii in apricots and almonds ..... E. E. WILSON 506

Effects of extract of western red-cedar heartwood on certain wood-decaying fungi in culture.
CHESTER M. SOUTHAM AND JOHN EHRLICH 517

Phytopathological Notes 525

The bulb or stem nematode on alfalfa, sweet clover, and white clover ..... OLIVER F. SMITH AND MERLIN W. ALLEN

Phoma terrestris in the roots of mature maize plants.
HELEN JOHANN

Scolecospores in Diplodia macrospora ..... PAUL E. HOPPE

Plot technique for disease-control studies on fine turf.
F. BEN STRUBLE

A method for obtaining single-pore cultures of Agaricus campestris ..... D. J. DEZEEUW

Host-parasite relationship in rust-infected oats.
HARRY B. HUMPHREY AND JEAN DUFRENOY

NOTE: The title of a paper **Leaf blight of corn** by C. W. ELLETT was inadvertently omitted from the Table of Contents of the May number.

VAPOR ACTION OF CERTAIN FUNGICIDAL MATERIALS PREPARED FOR DUSTING COTTON SEED[1]

S. G. Lehman

(Accepted for publication September 24, 1942)

In recent years certain commercial preparations having fungicidal properties have been widely recommended for dusting seeds before planting. Numerous workers have shown great reduction in smut of wheat (9), oats (10, 15), and barley (8) and marked improvement in cotton seedling emergence and survival (1, 4, 7, 11, 12, 14) as a result of treating seed with copper, zinc, and mercury dusts. The first dust preparation to be widely used on cotton seed was Ceresan. Ceresan, as prepared at present, contains 2 per cent of ethyl mercury chloride as its active ingredient. A second preparation, introduced for use in control of seed-borne parasites of cereals and since found to be very effective in control of fungi found on cotton seed, is sold under the name New Improved Ceresan. This preparation contains 5 per cent of ethyl mercury phosphate as its active ingredient. Another preparation of considerable merit for dust treatment of cotton seed is known to the trade as Sanoseed. This preparation is said by the manufacturer to contain not less than 2 per cent of ethanol mercury chloride.

Recommendations for seed treatment usually have emphasized the importance of using enough of the chemical and of thorough mixing of chemical and seed so that each seed becomes coated with particles of the disinfectant. With non-volatile disinfectants this would seem necessary. However, consideration of availability and cost of materials and of the toxic effect of the chemicals on the seed itself makes the use of small quantities desirable. A gaseous fumigant might have considerable advantage over one of low volatility in that the former, although used in small doses, could reach spores lodged in minute crevices impenetrable by fungicides in water solution.

When New Improved Ceresan is used for cotton, 1 and ½ oz. usually is recommended as the quantity to use for 1 bushel of fuzzy seed. In the writer's experiments in which this dust was applied in different quantities to cotton seed infested with the anthracnose fungus, *Glomerella gossypii*, and the pink boll rot fungus, *Fusarium moniliforme*, application of as little as ½ or ¼ ounce per bushel of seed usually has given nearly as great improvement in seedling emergence and survival as the use of larger quantities. It seems improbable that so little as ½ oz. of this dust containing only 0.025 oz. of active ingredient could by the methods employed be so uniformly distributed over 30 lb. of fuzzy seed as to bring a fungicidally effective dose in direct particle contact with more than a relatively small proportion of the spores on the seed. It was suggested that the effectiveness of this dust in

[1] Contribution from the Department of Botany, North Carolina Agricultural Experiment Station. Published with the approval of the Director as Paper No. 142 of the Journal Series.

small dosages is due to the volatility of the mercurial ingredient. It has been shown that certain microorganisms (5) and seed plants (6, 16) and human beings (3) may be harmfully affected by vapors arising from mercury or its compounds and that formaldehyde vapor (2) is an effective agent for control of fungi on seeds. With a seed like cotton, which normally has its surface covered with short lint, a volatile fungicide should have a marked advantage over one of no volatility. This paper gives an account of experiments made to test the vapor action of mercurial preparations used for treating cotton seed.

EXPERIMENTS WITH SEEDS

In these experiments both naturally infested and artificially inoculated seeds were used. All broken and visibly defective seeds were removed in order to reduce as much as possible the inaccuracies that might arise from inclusion of defective seeds. The sound seeds reserved for use were thoroughly mixed and then separated into equivalent lots for treatment. In making the treatments, a weighed quantity of the chemical preparation to be used was spread in a thin layer over the bottom of a large glass dish. Sufficient time was allowed for all dust to settle. The seeds were laid on wire racks above the chemicals. In some experiments the treatments were made in desiccator dishes with vapor-tight lids; in others, large 3-liter capacity culture dishes with well fitted but unsealed lids were used. Control lots were treated similarly, except for omission of the fungicide from the dishes. The treated seeds were germinated either in steamed sand in flats in the greenhouse or on paper toweling in large glass moist-chamber dishes in the laboratory. Fifty seeds were taken as the planting unit for each sublot, and each planting was replicated 4 or 8 times. The sublots of each test were randomized in the plantings. Other details of the treatments will be given as the various experiments are described.

In experiment 1 (Table 1), seeds naturally infested with *Glomerella gossypii* and *Fusarium moniliforme* were exposed to vapor from New Improved Ceresan. In experiment 2 (Table 1), both Ceresan and New Improved Ceresan were tested. Such an amount of each dust preparation was used as would give equivalent weights of the active ingredients. The seeds were germinated in steamed sand. In both experiments, the control lots gave a low percentage of disease-free plants, while the lot stored above Ceresan and New Improved Ceresan and the lot in which Ceresan was applied directly to the seed gave high percentages of disease-free seedlings. Obviously both Ceresan and New Improved Ceresan contain substances whose vapors are fungicidally active toward *G. gossypii* and *F. moniliforme* present on seed.

The treatments described above were made at laboratory temperatures which varied from 20° C. upward. It was of some interest to know whether similar effects would follow treatment at a lower temperature. Table 2 gives results of a test (Exp. 1) in which naturally infested seed was treated at a uniform temperature of 14° C. and at 28° C. The seeds were germinated

on paper toweling in large moist chamber dishes. This method of germination made it possible to identify the fungi present by direct examination of the diesased seedlings. *Fusarium moniliforme* and *Glomerella gossypii* were present on nearly all of the diseased seedlings. Seeds treated with vapors from New Improved Ceresan at 14° for 24 hours showed a higher percentage of germination and a much lower percentage of diseased seedlings than the untreated control. The 48- and 72-hour exposure periods gave disease control that was somewhat better than the 24-hour period, but not so good as that obtained by direct application of the dust to the seed. Exposure at 28° C. appears to have given somewhat better control of seedling diseases than at 14° C. Other similar tests not being presented here show good vapor action of New Improved Ceresan at 18° C. for exposure periods of 24 and 48 hours.

TABLE 1.—*Number and percentage of diseased seedlings arising from cotton seeds naturally infected with Glomerella gossypii and Fusarium moniliforme and subjected to vapors arising from Ceresan and New Improved Ceresan*

Experiment No.	Lot	Treatment	Seedlings free of disease	
			Number[a]	Percentage seeds planted
1	A	Control; stored 3 days in empty dish	1.6	3.2
	B	Stored 3 days over New Improved Ceresan[b]	47.4	94.8
	C	Control; stored 16 days in empty dish	2.6	4.2
	D	Stored 16 days over New Improved Ceresan[b]	47.5	95.0
		Difference required for significance, odds 99 to 1	3.6	7.2
2	A	Control; stored 2 days in dish without Ceresan	8.3	16.6
	B	Stored 2 days over 15 gms. of Ceresan[c]	34.4	72.8
	C	Stored 2 days over 6 gms. of New Improved Ceresan[b]	46.5	93.0
	D	Seed dusted with Ceresan[c]	46.1	92.2
		Difference required for significance, odds 99 to 1	2.6	5.2

[a] Mean of 8 replications of 50 seeds each.
[b] 5 per cent ethyl mercury phosphate.
[c] 2 per cent ethyl mercury chloride.

In other experiments vapor arising from Sanoseed was tested against *Glomerella gossypii*. Old seeds known to be free of natural infestation of *Fusarium moniliforme* were artificially inoculated with conidia of *G. gossypii*. The air-dry inoculated seeds were exposed to vapor from 5 g. of Sanoseed in large culture dishes, held at 3 constant temperatures. The results are given as Exp. 2 and 3 in table 2. Experiment 2 was germinated on paper toweling in moist chamber dishes at room temperature. A significantly smaller proportion of the seedlings became diseased from seeds, exposed to Sanoseed at 28° than at 5° C. However, this decrease appears to have been due to an adverse effect of drying the conidia on the seeds held at 28° C. The percentage of diseased seedlings from seeds exposed to Sanoseed at 38° is less by low odds than from seeds of the comparable untreated control.

TABLE 2.—*Effect of subjecting cotton seed naturally and artificially infested with G. gossypii to vapor from seed treatment preparations at different temperatures and for different periods of time*

Experiment No.	Treatment: Preparation used	Temperature °C.	Time hrs.	Germination as percentage seeds planted[a]	Diseased seedlings[b]: as percentage seeds planted	Diseased seedlings[b]: as percentage seeds germinated
1[d] [g]	Control, no fungicide used	Room		59.5	43.5	73.1
	New Imp. Ceresan,[j] vapor	14	24	74.0	12.5	16.9
	" " " "	"	48	88.5	4.5	5.1
	" " " "	"	72	82.5	4.0	4.8
	" " " "	28	48	82.0	2.0	2.4
	" " " dust on seeds	Room	[c]	79.5	0.0	0.0
	Diff. req. for signif.; odds 99 : 1			14.1	5.7	
2[d] [h]	Control, no fungicide used	28	72	87.0	70.5	81.0
	Sanoseed,[i] vapor	5	"	88.0	81.5	92.6
	" "	28	"	84.5	69.0	81.7
	" "	38	"	74.5	56.0	75.2
	Control, no fungicide used	38	"	74.5	62.5	83.9
	Diff. req. for signif.; odds 99 : 1			10.2		10.2
3[e] [h]	Control, no fungicide used	18	72	6.5[f]	6.5	100.0
	Sanoseed, vapor	18	"	17.5	16.5	94.3
	Control, no fungicide used	28	"	11.0	11.0	100.0
	Sanoseed, vapor	28	"	6.5	6.5	100.0
	Control, no fungicide used	38	"	11.0	10.5	95.5
	Sanoseed, vapor	38	"	15.5	15.5	100.0
	Ceresan,[k] vapor	18	"	83.5	0.0	0.0
	" "	28	"	88.0	0.0	0.0

[a] Percentage is based on 200 seeds, the number planted for each treatment.
[b] *Glomerella gossypii* and *Fusarium moniliforme* in experiment 1, *G. gossypii* alone in experiments 2 and 3.
[c] Dust applied to seeds about 1 hour before planting.
[d] Seeds germinated on paper in culture dishes.
[e] Seeds germinated in steamed sand in greenhouse.
[f] Figures represent per cent seeds emerged, total germination not determined on experiment 3.
[g] Seeds naturally infested with *G. gossypii* and *F. moniliforme*.
[h] Seeds free of *F. moniliforme* but artificially inoculated with *G. gossypii*.
[i] Contains 2 per cent of ethanol mercury chloride.
[j] Contains 5 per cent of ethyl mercury phosphate.
[k] Contains 2 per cent of ethyl mercury chloride.

In experiment 3 (Table 2) the seeds were planted in steamed river sand in the greenhouse. Total germination was not determined but emergence and disease records were taken. No vapor action of Sanoseed against *Glomerella gossypii* was indicated. Most of the seedlings died before emergence. The small proportion that did emerge died soon afterward or showed pronounced anthracnose lesions on the stems. Vapor from Ceresan, however, gave high seedling emergence and complete freedom from disease. These results (Exp. 2 and 3), which are supported by a preliminary experiment not given here, indicate that vapor action by Sanoseed is only feebly if at all effective at ordinary temperatures.

Old seeds or seeds that have been improperly stored often are found infested with and susceptible to injury by such molds as *Rhizopus nigricans*, *Cephalothecium roseum*, and *Aspergillus* sp., fungi not usually regarded as important parasites of cotton seedlings. Seed of this character, even though free of the more virulent parasites, *Glomerella gossypii* and *Fusarium moniliforme*, is often greatly improved in respect to seedling emergence by dusting with a mercury fungicide before planting in the field. A preliminary experiment with old seeds exposed to vapor of New Improved Ceresan indicated considerable reduction in seedling mortality from such treatment. To establish this point additional experiments were made using seed approximately 5 years old and known to be infested by and susceptible to *R. nigricans*. A brown-spore and black-spore species of *Aspergillus* were also on the seeds. In the course of these experiments, the temperature, period, and manner of exposure were varied somewhat, and several dust preparations were used. The seed was germinated on paper toweling in large moist-chamber dishes in order better to observe the fungi developing on the seed and seedlings. Two hundred seeds were germinated for each treatment. After germination began, records were taken at daily intervals, and diseased seedlings were removed as soon as the causal fungi could be recognized. Certain details and the results of these treatments are given in table 3.

In experiment 1 (Table 3), molds, chiefly Rhizopus, destroyed 43.5 per cent of the seeds of the control, either before or soon after germination. Storage in vapor above ethyl mercury phosphate greatly reduced the percentage of moldy and damaged seedlings and increased the percentage of uninjured seedlings. Treatment at 28° C. gave markedly better results than at 5° C. Application of the dust directly to the seeds entirely inhibited growth of the seed-molding fungi.

In experiments 2 and 3 (Table 3), several seed-treatment preparations were used and such an amount of each preparation was stored in the dishes with the seeds as to give an equal weight of the active ingredient for each preparation tested. Thus, if the concentrations of active ingredient as given by the manufacturer were correct, the seeds in each treated lot were exposed to vapors from 0.1 g. of the chemical ingredient. Best results from the vapor treatments were obtained by use of the preparation containing ethyl mercury chloride. Not only were fewer seeds destroyed by mold fungi

TABLE 3.—*Effect of subjecting cotton seed infested with Rhizopus, Aspergillus and other mold fungi to vapors arising from seed-treatment preparations containing mercury in organic combination*

Experiment No.	Treatment					Seeds moldy; not germinating, or radicles diseased soon after germination[a]	Seeds moldy; germinating, radicles not injured[b]	Seeds germinating, radicles not injured[c]
	Active ingredient and percentage in preparation used	Amount of preparation used	Time	Temperature	Receptacle			
		Grams	*Hours*	*° C.*		*Per cent*	*Per cent*	*Per cent*
1	Control; no chemical	....	72	Room	Paper bag	43.5	18.0	52.0
	Ethyl mercury phosphate, 5 per cent	5	72	5° C.[e]	Covered dish	27.5	26.5	73.5
	Ethyl mercury phosphate, 5 per cent	5	72	28° C.[e]	" "	1.5	0.0	91.0
	E-M-P dust on the seed, 5 per cent	[d]	72	Room	Paper bag	0.0	0.0	91.5
	Diff. req. for signif., odds 99 : 1	....	....	.....	.....	10.7	.....	.....
2	Control; no chemical	....	50	22[f]	Paper bag	47.5	4.5	50.5
	Ethyl mercury chloride, 2 per cent[g]	5	50	"	Covered dish	3.5	0.5	94.5
	Ethyl mercury phosphate, 5 per cent[h]	2	50	"	" "	24.5	16.0	75.0
	Ethyl mercury borate, 5 per cent[i]	2	50	"	" "	19.5	13.5	80.0
	Ethyl mercury iodide,[i] 5 per cent	2	50	"	" "	7.5	35.0	91.5
	Ethanol mercury chloride, 2 per cent	5	50	"	" "	52.0	2.0	48.0
	Ethanol-M-C dust on seed, 2 per cent[j]	[d]	18	"	Paper bag	3.5	0.0	96.0
	Diff. req. for signif., odds 99 : 1	....	.....	.....	.....	16.8	.....	.....
3	Control; no chemical	....	73	26[f]	Paper bag	38.0	10.0	47.0
	Ethyl mercury chloride, 2 per cent	5	73	"	Covered dish	2.0	0.0	87.5
	Ethyl mercury chloride, 2 per cent	5	73	"	Sealed dish	2.0	1.0	86.5
	Ethyl mercury phosphate, 5 per cent	2	73	"	Covered dish	17.5	27.5	73.5
	Ethyl mercury phosphate, 5 per cent	2	73	"	Sealed dish	16.5	40.0	69.5
	Ethyl mercury borate, 5 per cent	2	73	"	Covered dish	20.5	27.5	69.5
	Ethyl mercury iodide, 5 per cent	2	73	"	" "	3.5	43.0	86.5
	Ethanol mercury chloride, 2 per cent	5	73	"	" "	43.5	9.5	49.0
	E-M-borate dust on seed, 5 per cent	[d]	73	"	Paper bag	0.0	0.0	87.5
	Diff. req. for signif., odds 99 : 1	....	.....	.....	.....	14.8	.....	.....

[a] *Rhizopus nigricans* on most of the seeds; a brown-spore *Aspergillus* second in importance, *Cephalothecium roseum* and *Penicillium* present but of minor importance.

[b] A black-spore *Aspergillus*, apparently entirely non-parasitic, was the chief fungus present on the seed coats.

[c] Includes percentages given in column (b) but none in column (a).

[d] All that will adhere to seed after shaking lightly on a screen.

[e] In constant temperature chambers.

[f] In warm room, the mean temperature for the period of exposure.

[g] Ceresan.

[h] New Improved Ceresan.

[i] Experimental preparation, no established trade name.

[j] Sanoseed.

(*Rhizopus nigricans* and the brown-spore *Aspergillus*), but also the vapor almost completely prevented growth of the non-parasitic black-spore *Aspergillus* on the coats of germinating seedlings. Ethyl mercury iodide was second in respect to effectiveness of its vapors in preventing *Rhizopus* and the brown-spore *Aspergillus* from destroying seeds and seedlings. However, in control of the black-spore *Aspergillus* on the coats of germinating seeds, the ethyl mercury iodide preparation was least effective of any tested. Some selective action between fungi and chemical seems to be indicated. Ethyl mercury phosphate and ethyl mercury borate preparations were each about equally effective in respect to the fungicidal action of their vapor. Each also was significantly less effective than ethyl mercury chloride against all fungi on the seeds. The least effect on seed-destroying fungi was obtained from vapor from ethanol mercury chloride. This material is obviously of low volatility. The same material applied directly to the seeds, however, gave good control of fungi on the seeds.

In experiment 3 (Table 3), comparisons were made between treatments in dishes having ground glass lids sealed with petrolatum and in culture dishes having well fitted but unsealed lids. No advantage was found in the use of a sealed dish as compared to a covered, unsealed dish. Apparently the quantities of material used (0.1 g. of active ingredient) was great enough to maintain an atmosphere approximately saturated with chemical vapor in the covered, unsealed dishes.

EXPERIMENTS WITH CONIDIA

In addition to experiments with infested cotton seed other experiments were carried out in which conidia of *Glomerella gossypii* were exposed to vapors from various chemical preparations. A culture of the fungus isolated from a diseased cotton seedling and known to be pathogenic was grown on potato-dextrose agar in Petri dishes until an abundance of spores had formed. The surface of the dishes was rinsed with water to get a heavy suspension of conidia. Two 3-mm. loopfuls of this conidial suspension were placed on small squares of sterile filter paper in sterile Petri dishes. The Petri dishes containing the inoculated filter paper squares were placed in a cupboard with the lids removed until the paper and spores had become air-dry. The dry inoculated papers were exposed to the vapors of the chemicals in the manner described above for exposure of seeds; *i.e.*, by placing an open Petri dish in a closed culture dish over the chemical being tested. In some instances instead of exposing inoculated papers, the conidia were placed on the papers after exposure to the chemicals. Glass cover slips were substituted for the paper in some experiments. After the treatment these were placed on water agar containing 0.1 per cent dextrose to determine if the treated conidia were still viable. For convenience the paper squares and glass cover slips with conidia will be referred to as "cultures."

Preliminary tests indicated that conidia of *Glomerella gossypii* on paper squares could be killed by exposure to vapor from 2 g. of New Improved

TABLE 4.—*Effect of exposing air-dry conidia of Glomerella gossypii on filter paper to vapor emanating from preparations containing mercury in organic form*

Exp. No.	Treatment: Active ingredient and percentage in preparation used	Amount of preparation used g.	Length of gassing period (hours): 0	1	2	3	4	6	12	24
			Number of cultures alive out of 5 gassed							
1	Control	0	5							
	Ethyl mercury phosphate, 5 per cent	2.0						0	0	0
	Ethyl mercury phosphate, 5 per cent	1.0								0
	Ethyl mercury phosphate, 5 per cent	0.5								0
	Ethyl mercury phosphate, 5 per cent	0.25								0
2	Control	0	5							
	Ethyl mercury phosphate, 5 per cent	2.0		1	0		0	0		
3	Control	0	5[a]							
	Ethyl mercury chloride, 2 per cent	5		0	0	0	0			
	Ethyl mercury phosphate, 5 per cent	2		0	0	0	0			
	Ethyl mercury borate, 5 per cent	2		5	2	1	0			
	Ethyl mercury iodide, 5 per cent	2		1	0	0	0			
4	Control	0	5[b]							
	Ethyl mercury chloride, 2 per cent	5		0	0	0	0			
	Ethyl mercury phosphate, 5 per cent	2		2	0	0	0			
	Ethyl mercury borate, 5 per cent	2		5	2	0				
	Ethyl mercury iodide, 5 per cent	2		0	0	0	0			

[a] Mean of 4 control plates of 5 cultures each.
[b] Mean of 2 control plates of 5 cultures each.

Ceresan for a period of 24 hours. Table 4 gives results from other experiments using different seed treatment preparations, different weights of certain of the preparations, and different exposure periods. In experiments 1 and 2 as little as 0.25 g. of the ethyl mercury phosphate preparation provided sufficient vapors to prevent growth of cultures after 24 hours of gassing. Exposure as short as 2 hours to 2 g. resulted in failure of all cultures to grow.

Comparative tests of vapor action of 4 seed-treatment preparations were made in experiments 3 and 4 (Table 4). Such a quantity of each preparation was taken as would put an equivalent amount (0.1 g.) of the active ingredient in the gassing chambers (large, unsealed culture dishes). Treatments were continued from periods of 1, 2, 3, and 4 hours at a temperature of 28° C. No cultures grew after 1 hour's exposure to the preparation containing ethyl mercury chloride. The preparations containing ethyl mercury phosphate and ethyl mercury iodide were apparently somewhat less effective than ethyl mercury chloride. Of the 4 preparations applied ethyl mercury borate was least effective as a volatile agent, more than 2 hours' exposure being required to inhibit growth of all cultures by this material.

Other experiments were made in which air-dry conidia were gassed at different temperatures by vapors from ethyl mercury chloride, ethyl mercury phosphate, and ethanol mercury chloride (Table 5). At all temperatures used (5, 18, and 28° C.) vapors from the ethyl mercury chloride completely inhibited growth of the filter-paper cultures. The ethyl mercury phosphate preparation was somewhat less effective, only partly inhibiting growth for the 2- and 4-hour treatment periods at 5° C. Growth was prevented completely when treatments were made at 28° C. Ethanol mercury chloride was much less effective in vapor action than the other materials used. Growth occurred from all cultures except one exposed to this material.

In the experiments described above, growth of the conidia exposed to vapors of certain organic mercury compounds was inhibited. Assuming that the chemical vapors condense on the conidia and paper and become fungicidally active only when the cultures are put on moist media for germination, one might expect those vapors that had condensed on the conidial cultures from a saturated atmosphere in a given period of exposure would in a like period harmlessly volatilize from the cultures when they were moved to open air. Table 6 gives results of exposing air-dry conidia on filter paper to vapors of 4 organic mercury preparations for 1, 3, 4, and 24 hours and subsequently moving them to open air to permit escape of the material that may have condensed on them. No growth occurred after 24 hours' exposure to any of the 4 chemicals, even though such exposure was followed by a 24-hour period of airing to permit volatilization and dissipation of condensed vapors adhering to the surface of paper and conidia. With ethyl mercury chloride and ethyl mercury phosphate, growth

of all cultures was inhibited by 3 hours of gassing; but 24 hours of subsequent airing failed to remove the inhibiting agent. A period of airing considerably longer than the period of exposure was required to remove the inhibiting chemical from the cultures; and increasing the period of exposure of the cultures to the vapors increased the difficulty of freeing them of the chemicals by airing. It appears from these tests that the chemicals either penetrate and kill the dry conidia or in some way become

TABLE 5.—*The effect of exposing air dry conidia of Glomerella gossypii on filter paper at different temperatures to vapor from 3 preparations containing organic mercury*

Treatment			Number of cultures alive out of 5 treated	
Active ingredient in preparation used	Temperature	Time		
	°C.	*Hours*	*Expt. 1*[d]	*Expt. 2*[d]
Control, not gassed	Room		5	5
Ethyl mercury chloride, 2 per cent[a]	5	2	0	0
" " " " " "	"	4	0	0
" " " " " "	28	2	0	0
" " " " " "	"	4	0	0
Ethyl mercury phosphate, 5 per cent[b]	5	2	2	2
" " " " " "	"	4	1	1
" " " " " "	28	2	0	0
" " " " " "	"	4	0	0
			Expt. 3[d]	*Expt. 4*[d]
Ethanol mercury chloride, 2 per cent[c]	18	72	5	5
Control, not gassed	"	"	5	5
Ethanol mercury chloride, 2 per cent	28	"	5	5
Control, not gassed	"	"	5	5
Ethanol mercury chloride, 2 per cent	38	"	5	4
Control, not gassed	"	"	5	5
Ethyl mercury chloride, 2 per cent	18	"	0	0
" " " " " "	28	"	0	0
			Expt. 5[e]	
Ethanol mercury chloride, 2 per cent	28	24	5	
" " " " " "	"	48	5	
" " " " " "	"	72	5	
Control, not gassed	"	"	5	

[a] Ceresan; 5 grams in each gassing dish.
[b] New Improved Ceresan; 2 grams in each dish.
[c] Sanoseed; 5 grams in each dish.
[d] Gassed in large, unsealed culture dishes.
[e] Gassed in sealed desiccator dishes.

attached to the cultures (conidia and paper) so firmly as to considerably retard release when the cultures are removed to open air.

A number of experiments were made in which conidia of *Glomerella gossypii* were put on filter-paper squares after such squares had been gassed. These experiments showed that ethyl mercury chloride and phosphate vapor condensed on the paper alone in sufficient quantity to stop growth of conidia subsequently placed on the papers and that removal of the inhibiting chemicals required a period of airing considerably longer than the period of gassing in which the chemical vapors condensed on the papers. Table 7 gives

the results of an experiment in which the effects of adding the conidia to the papers before or after gassing are compared. Two series of papers were exposed for 1 hour to vapors of ethyl mercury chloride. In series A, the conidia of *G. gossypii* were put on papers and dried. Then the gassed cultures were aired as shown. In series B, the conidia were put on the papers

TABLE 6.—*Effect of airing after exposing air dry conidia of Glomerella gossypii on filter paper to vapor emanating from preparations containing organic mercury*

Exp. No.	Active ingredient in preparation used[a]	Period of gassing	Period of gassing	Cultures alive out of 5 tested
		Hours	*Hours*	*Number*
1	Control, not gassed			5
	Ethyl mercury phosphate, 5 per cent	24	0	0
	" " " " " "	"	24	0
	Ethyl mercury chloride, 2 per cent	"	0	0
	" " " " " "	"	24	0
	Ethyl mercury borate, 5 per cent	"	0	0
	" " " " " "	"	24	0
	Ethyl mercury iodide, 5 per cent	"	0	0
	" " " " " "	"	24	0
2	Control, not gassed			5
	Ethyl mercury phosphate, 5 per cent	1	0	0
	" " " " " "	1	24	5
	" " " " " "	3	0	0
	" " " " " "	3	24	0
	Ethyl mercury chloride, 2 per cent	1	0	0
	" " " " " "	1	24	1
	" " " " " "	3	0	0
	" " " " " "	3	24	0
	Ethyl mercury borate, 5 per cent	1	0	5
	" " " " " "	1	24	5
	" " " " " "	3	0	1
	" " " " " "	3	24	5
	Ethyl mercury iodide, 5 per cent	1	0	0
	" " " " " "	1	24	5
	" " " " " "	3	0	0
	" " " " " "	3	24	4
3	Control, not gassed			5
	Ethyl mercury chloride, 2 per cent	4	0	0
	" " " " " "	4	25	0
	Ethyl mercury phosphate, 5 per cent	4	0	0
	" " " " " "	4	25	0
	Ethyl mercury borate, 5 per cent	4	0	0
	" " " " " "	4	25	5
	Ethyl mercury iodide, 5 per cent	4	0	0
	" " " " " "	4	25	1

[a] The quantity of each preparation used was such as to give an equivalent quantity of active ingredient in each dish.

after the latter had been gassed and aired. Both series were gassed concurrently in the same chamber for 1 hour at 25° C., and then aired concurrently, in the same location, at 25° C. For each treatment in each series, 10 cultures, 5 in each of 2 Petri dishes, were tested for viability. As indicated by lot 2 (Table 7), gassing the papers before or after adding conidia prevented growth of all cultures if the gassed papers were not aired before being placed

on agar. Airing for 4 hours subsequent to gassing, enabled 1 culture of series A and 7 of series B to grow. Airing for 8 hours subsequent to gassing permitted only 2 cultures of series A to grow, while 10 grew in series B. Airing 16 or 24 hours permitted only 8 cultures of series A to grow, while 10 grew in series B. The colonies in lots 4, 5, 6 from cultures of series A were noticeably smaller than those from series B, due to a slowing up of germination by the chemical or to a reduction of the number of conidia

TABLE 7.—*Effect of airing culture papers exposed to vapors of ethyl mercury chloride before or after adding conidia of Glomerella gossypii*

Lot No.	Treatment		Number of cultures alive out of 5 tested			
		Period of airing hrs.	Series A. Papers[a] gassed after adding conidia		Series B. Papers[b] gassed before adding conidia	
			Plate 1	Plate 2	Plate 1	Plate 2
1a	Not gassed	25	5[l]	5[l]	.....	.....
1b	Not gassed	0	.....	.....	5[l]	5[l]
2	Gassed	0	0	0	0	0
3	Gassed	4	0	1[s]	2[s]	5[s]
4	Gassed	8	2[s]	0	5[l]	5[l]
5	Gassed	16	5[s]	3[s]	5[l]	5[l]
6	Gassed	24	5[s]	3[s]	5[l]	5[l]

[a] Except lot 1a; [b] except lot 1b; [l] colonies large; [s] colonies small.

germinating in series A. In lot 3, aired for 4 hours, colonies were small in both series A and B. No colony size differences were evident between the controls for the two series. Since fewer cultures grew and colonies were smaller in series A, in which both paper and conidia were gassed, than in series B, in which only the paper substratum was gassed, it appears that the chemical used adheres somewhat more tenaciously to conidial walls than to the filter paper.

The foregoing tests show that vapors of the chemicals used adhere to filter paper in quantities lethal to *Glomerella gossypii* and that the chemicals adhere for considerable periods of time after exposure to vapor-free air. An effort was made to learn if treatment of conidia deposited on glass would have a different effect than treatment of conidia on paper. Conidia of *G. gossypii*, dried down on sterile filter paper and on glass cover slips, were gassed by ethyl mercury chloride and ethyl mercury phosphate in sealed desiccator dishes at 3 temperatures for 2 time intervals (Table 8). Growth of cultures on paper was inhibited at all exposure periods by both chemicals at 13° and 28° C. and also at 5° C. by ethyl mercury chloride. On glass no inhibition occurred, except by ethyl mercury chloride at 5° C. and 4 hours' exposure.

In other experiments, conidia on glass cover slips were exposed to ethyl mercury chloride and ethyl mercury phosphate for periods of 12 or more hours in sealed dishes (Table 9). In experiment 1, the conidia were dried on cover slips for a uniform period of time before gassing. In experiments

2 and 3 the conidia were dried for short and long periods of time. It was expected that differences in moisture content of the conidial walls might result from short and long drying periods and that this would in turn effect absorption of the chemical vapors and produce demonstrable differences in viability of the treated cultures. Such results did not follow. Conidia dried in experiment 3 for so short a period as 40 minutes, still were able to germinate after being gassed as long as 48 hours on glass. In other experiments noted above, exposure of conidia on filter paper for 12 or 24 hour periods to vapors of these chemicals resulted invariably in inhibited growth.

TABLE 8.—*Effect of exposing air dry conidia of Glomerella gossypii on filter paper and on glass coverslips to vapors emanating from preparations containing organic mercury compounds at different temperatures*

Active ingredient in preparation used	Temperature during treatment[a] ° C.	Period of gassing hours	Number of cultures alive out of 5 gassed on	
			Paper	Glass
Control, not gassed	Room		5	5
Ethyl mercury chloride, 2 per cent[b]	5	2	0	5
" " " " " "	5	4	0	0[d]
" " " " " "	13	2	0	5
" " " " " "	13	4	0	5
" " " " " "	28	2	0	5
" " " " " "	28	4	0	5
Ethyl mercury phosphate, 5 per cent[c]	5	2	5	5
" " " " " "	5	4	5	5
" " " " " "	13	2	0	5
" " " " " "	13	4	0	5
" " " " " "	28	2	0	5
" " " " " "	28	4	0	5

[a] Treatments were made in desiccator dishes having lids sealed with petrolatum.
[b] Ceresan; 5 g. in each dish.
[c] New Improved Ceresan; 2 g. in each dish.
[d] Microscopic examination showed many conidia on each coverglass, but none had germinated.

The results presented in tables 8 and 9, taken in conjunction with data presented in preceding tables, show that conidia on glass are less readily inhibited from growth by exposure to vapors of the chemicals used than conidia on paper. Seemingly, in the foregoing experiments the chemical vapors had as great opportunity to condense on, enter and kill dry conidia gassed on glass as when gassed on paper. Apparently failure of the paper cultures to grow was due chiefly to action of vapor condensed on and held in the paper and later absorbed by the conidia when placed on moist agar. The chemicals failed to adhere to glass in the same quantities as to paper. The experiments with seeds (Tables 1, 2, 3) indicate that cotton lint may hold the condensed chemical vapors much as does filter paper.

In preliminary tests, cultures of *Glomerella gossypii* growing on moist potato-dextrose agar in Petri dishes were exposed to vapors of ethyl mercury chloride (Ceresan) in sealed dishes. Conidia thus gassed failed to grow when transferred to fresh nutrient agar. In other tests cultures of *G.*

TABLE 9.—*Effect of exposing dry conidia of Glomerella gossypii on glass cover slips to vapors emanating from preparations containing organic mercury*

Active ingredient in preparation used	Hours of gassing	Number of cultures alive out of 5 or 10 gassed		
		Exp. 1[a]	Exp. 2[bc]	Exp. 3[def]
Ethyl mercury chloride, 2 per cent	12	4		
" " " " "	24	3	5 – 10	5 – 5 – 5
" " " " "	48	...	... .	5 – 5 – 5
" " " " "	72	...	... .	 4
Ethyl mercury phosphate, 5 per cent	12	4	... .	... ...
" " " " "	24	5	10 – 10	... ...
Control[g]	0	5	10 – 10	5 – 5 – 5

[a] Conidia dried on cover slips 7 or 8 days before gassing. Five cultures gassed for each treatment.

[b-c], Conidia of [b] and [c] from same culture, gassed concurrently in same gassing chamber: [b] dried 4 hr., [c] dried 28 hr. before gassing. Ten cultures gassed each treatment combination.

[d-e, f]. Conidia of [d] dried on cover slips 40 minutes (until all macroscopically visible water had evaporated), [e] dried 1 hr. 40 min., [f] dried 24 hr. before gassing. Five cultures gassed for each treatment combination.

[g] Not gassed; stored in empty dish until end of longest gassing period used in the experiment.

gossypii were allowed to dry in a drying cabinet for various periods of time before exposure to vapors of the chemical. At the end of the gassing period, conidia, and in some tests conidia and pieces of agar substratum, were removed to nutrient agar. Details and results of some of these tests are given in table 10. The gassed conidia failed to grow, even though the gassing was done after the cultures had been permitted to become completely air dry. Enough of the chemical was taken up from the vapors and retained by the air-dry conidia alone to prevent subsequent growth.

TABLE 10.—*Effect of vapors of ethyl mercury chloride on conidia of Glomerella gossypii growing on nutrient agar in Petri dishes*

Exp. No.	Treatment of fungus	Estimated percentage water in substratum	Fungus alive in 10 transfers of	
			Conidia only	Substratum and conidia
1	Not dried, gassed	95	2	0
	Dried 24 hr., gassed	70	0	0
	Dried 48 hr., gassed	20[a]	0	0
	Dried 66 hr., gassed	15[a]	0	0
	Dried 48 hr., not gassed	20[a]	10	10
2	Dried 55 hr., gassed	15[a]	0	
	Dried 55 hr., not gassed	15[a]	10	
3	Dried 66 hr., gassed	[b]	0	
	Dried 66 hr., not gassed	[b]	10	
4	Not dried, gassed	95	0	0
	Dried 72 hr., gassed	[b]	0	0
	Dried 72 hr., not gassed	[b]	10	10

[a] Not completely air dry; cuts like rubber, but readily removed from dish.

[b] Completely air dry, difficult to remove pieces of substratum from dish.

TESTS WITH OTHER CHEMICALS

In the course of the work herein reported, conidia on filter paper squares were exposed to vapors that might arise from a number of chemicals other than those discussed above. The results from the tests with these preparations are given in table 11. Little or no vapor action was found for Semesan, creosote dust, benzol, benzine, paradichlorobenzene, or picric acid. Vapors from alkyl mercuric acetylene were partly effective in killing the conidia. This effectiveness is considerably enhanced by addition of urea. Leytosan, a preparation containing mercury in combination with phenol and urea, was slightly less effective than alkyl mercuric acetylene plus urea. A high proportion of the conidia were killed by vapors of 154–6B and trioxymethane. The latter did not readily evaporate from the filter paper that carried the conidia and probably would act in a similar manner on the short fibers on cotton seed. Vapor of chloropicrin killed all cultures gassed for 72 hours but only part of those gassed 24 hours. Ethyl ether was not effective at 24 hours' exposure, but prevented growth of all cultures gassed for 72 hours.

TABLE 11.—*Effect of exposing air-dry conidia of Glomerella gossypii on filter paper to vapors emanating from various chemical preparations*

Preparation and/or active ingredient therein	Period of gassing hours	Number cultures alive/number treated		
		Control, cultures not gassed	Cultures gassed	
			Not aired after gassing	Aired after gassing
Semesan (hydroxymercurochlorophenol), 30 per cent	24	10/10	10/10	
" " "	72	5/5	5/5	
Alkyl mercuric acetylene[a]	24	10/10	8/10	
" " "	72	10/10	5/10	
Alkyl mercuric acetylene plus urea[b]	24	5/5	0/5	
" " " " "	72	10/5	0/10	
154–6B[d]	28	10/10	0/10	
Leytosan (Phenolmercuricurea)	24	10/10	3/10	
" "	72	10/10	0/10	
Creosote dust (5 per cent creosote[c])	24	15/15	15/15	
" " " " " "	72	5/5	5/5	
Chloropicrin	24	10/10	6/10	
"	72	5/5	0/10	
Benzol C.P.	24	10/10	10/10	
" "	72	5/5	5/5	
Benzine (Petroleum ether) C.P.	24	10/10	6/10	
" " " "	72	5/5	5/5	
Ethyl ether C.P.	24	10/10	10/10	
" " "	72	5/5	0/5	
Para Bacea (Paradichlorobenzene)	24	10/10	10/10	
" " "	72	5/5	5/5	
Trioxymethane U.S.P.	24	10/10	2/10	0.5
" "	72	5/5	0/5	
Pierin acid crystals	24	5/5	5/5	
" " "	72	5/5	5/5	

[a] "M" compound, supplied by Chicago Development Laboratory, described as "insoluble."

[b] Supplied by Chicago Development Laboratory, described as "soluble."

[c] Supplied by Heyden Chemical Corporation, New York.

[d] Supplied by American Cyanamid and Chemical Corporation.

DISCUSSION

The experiments described in this paper show that cotton seeds infested with certain fungi are greatly benefited by confinement for a time in an atmosphere containing vapors emanating from certain organic mercury preparations. Likewise, conidia of *Glomerella gossypii* on filter paper were prevented from germination by suitable exposure to vapor from these materials. Preparations containing ethyl mercury chloride and ethyl mercury phosphate were much more effective in vapor action than similar preparations containing ethanol mercury chloride, and hydroxymercurochlorophenol, while ethyl mercury borate was intermediate in effectiveness. This difference appears to be correlated with volatility of the active materials. Ethyl mercury chloride and ethyl mercury phosphate are characterized by considerable volatility, while ethanol mercury chloride and hydroxymercurochlorophenol are relatively non-volatile at ordinary temperatures, and ethyl mercury borate is intermediate in this respect. In field tests with cotton seed dusted with preparations of ethanol mercury chloride and hydroxymercurochlorophenol, these 2 relatively non-volatile chemicals usually have been less beneficial than preparations containing the more volatile salts, ethyl mercury chloride or ethyl mercury phosphate.

The adherence of vapors of ethyl mercury chloride and ethyl mercury phosphate to filter paper and to conidia after removal from the gassing chambers to open air may be due to some physical bond with the substance of the paper or conidial walls or to diffusion into and retention by submicroscopic pores in the paper and conidial walls. After a time the vapors escape, thus removing inhibition to germination of the conidia; but the time required for release of the chemicals is considerably longer than the time for fixation of the vapors in amounts necessary to stop germination. After longer periods of gassing, longer periods of airing were required to release the vapors and permit germination of the conidia, indicating more complete saturation of the paper and conidia. Apparently storage of seed for several days after dusting with these materials should increase the effectiveness of the treatment. Seeds that have escaped direct particle contact with the chemical applied to a given seed lot, are effectively fumigated by vapor from particles on adjacent seeds. The vapor becomes fixed to the lint and to the fungi on the seed in such fashion as to be retained for a considerable period of time after removal from confinement.

Wolf and his coworkers (13) state that vapors of benzol and paradichlorobenzene effect control of the downy mildew fungus, *Peronospora tabacina*, in tobacco beds, by solution in the water on and within plant tissues and in constituents of the living cells of the tobacco plant and of the mycelium of the fungus. In certain of the experiments made in connection with work reported in this paper, it was observed that growth of the cultures gassed with ethyl mercury chloride after putting the conidia on the paper squares was more readily prevented than when the papers were gassed before adding the conidia. Thus it seemed that during the gassing period the vapors may have entered through the dry walls of the conidia in suffi-

cient quantity to inactivate their protoplasts. Since many of such cultures grew, however, after removal to open air for a suitable period of time, it appears that the chemical vapors were deposited on but did not enter the conidia until they were placed on moist agar for germination. The technique employed in these tests is not adequate to determine whether or not some of the conidia may have been killed by penetration of the dry walls by the chemicals during the gassing period.

Concentration of vapors in a confined atmosphere above exposed surfaces is dependent on temperature. Vapor action, inhibitory of spore germination, was observed for ethyl mercury chloride and ethyl mercury phosphate at temperatures as low as 5° C. and as high as 28° C. This well covers the range of weather temperature that would exist between the usual times of treating and planting cotton seed. Efficient vapor action was not observed for ethanol mercury chloride at any temperature tried. This may be basic to the somewhat poorer results obtained from ethanol mercury chloride in field tests in which this material has been compared with ethyl mercury chloride and ethyl mercury phosphate.

In the foregoing tests it has been assumed that the lethal vapors were those of the substances reported by the manufacturer to be the principal active ingredient in the preparations tested. However, since the mercury preparations used in most of the experiments were of commercial manufacture and were not made by mixing chemically pure salts with the diluents, it is recognized that minor percentages of other mercury compounds may have been present.

SUMMARY

The commercial seed treatment preparations sold as Ceresan and New Improved Ceresan and containing ethyl mercury chloride and ethyl mercury phosphate, respectively, as the chief active ingredient emit fungicidally active vapor. When cotton seeds were confined with this vapor, the vapor condensed on the seeds in sufficient quantity to inhibit growth of seed-infesting fungi. Similarly, vapor from preparations containing ethyl mercury borate and ethyl mercury iodide were found to act as a fumigant fungicide for cotton seeds.

Mercurial vapor from the 4 preparations mentioned above was lethal to conidia of *Glomerella gossypii* exposed on filter paper and on mycelium in Petri dishes. The vapor was retained by the paper and the conidia in quantity lethal to the conidia for considerable periods of time after removal to open air. The chemical appeared to be retained more tenaciously by conidia than by filter paper. In order of decreasing effectiveness of their vapor action against fungi on seeds and filter paper, these 4 chemicals rank as follows: ethyl mercury chloride, ethyl mercury iodide, ethyl mercury phosphate, and ethyl mercury borate, respectively.

Sanoseed, containing ethanol mercury chloride, gave good control of seed-infesting fungi when applied directly to cotton seed, but little or no beneficial effect was found when infested seed or conidia of *G. gossypii* on filter paper were exposed to its vapor at temperatures of 38° C. or lower.

Ethanol mercury chloride is of low volatility at temperatures such as those to which treated cotton seed would be exposed.

By reason of the volatile nature of ethyl mercury chloride and ethyl mercury phosphate and their property of condensing on and adhering to cotton seed and seed-infesting fungi, very small ratios of quantity of chemical to quantity of seed are effective. Vapor from seeds carrying bits of the chemical pass to and condense on adjacent seeds that may have received none of the chemical in the dusting operation. Vapor action of ethyl mercury chloride and ethyl mercury phosphate is effective in the range of temperatures (5° to 38° C.) likely to obtain between the dates of treating and planting cotton seed.

Tests of Semesan (30 per cent hydroxymercuro-chlorophenol), creosote dust (5 per cent creosote), benzol, benzine (petroleum ether), para bacca (paradichlorobenzene), and picric acid, showed no inhibitory effect of their vapors toward air-dry conidia of *G. gossypii*. Marked reduction or complete prevention of germination of conidia of this fungus was effected by vapors of alkylmercuricacetyleneurea, leytosan (phenomercuricurea), 154–6B, chloropicrin, ethyl ether, and trioxymethane. Alkylmercuricacetylene was only partly effective as a fumigant fungicide.

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A DESTRUCTIVE VIRUS DISEASE OF SOUR CHERRY[1]

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For many years an unfruitful condition of sour cherry (*Prunus cerasus* L.), known to the growers as "yellow leaf" or "boarder tree," has been observed in Wisconsin. Similar or identical troubles have been reported by Stewart (21) and Gloyer and Glasgow (6) as a non-parasitic "yellow leaf" and by Rasmussen (17) as "physiological yellow leaf." Various possible causes of the trouble have been suggested, but convincing experimental evidence as to cause has been lacking. Observations that the malady was spreading in Wisconsin orchards led the writers in 1936 to underake studies that have shown that they are dealing with a virus disease, or possibly a complex of virus diseases. Brief reports on this work have been published (3, 4, 11, 12, 13, 14, 15). The present paper[2] reports the progress of this investigation from its beginning to the end of the season of 1940.

Cherry yellows is tentatively proposed (*cf.* 4) as the common name of this disease because of the type of leaf symptoms and the probability discussed herein that it is transmitted by leaf hoppers.

In the present state of knowledge of virus diseases of stone fruits, it is not possible to give a complete account of the etiology and symptom expression of cherry yellows or to define its relations to certain other virus diseases. The following account is to be regarded as a report of progress based chiefly on field experiments and observations extending over a 5-year period. In some respects, especially the identity of the virus or virus complex and the range of symptom expression, it is subject to modification or correction in the light of results of further studies now in progress.

A general review of literature relating to virus diseases of stone fruits seems unwarranted here, since, for present purposes, the field has been sufficiently covered by others (1, 2, 9).

Since this paper was drafted, several articles on the disease under consideration have appeared (9, 10, 18, 19). These will be referred to later in connection with topics on which they bear.

SYMPTOMS

The leaf symptoms described below are characteristic of the disease as the writers have seen it develop naturally or after transmission by budding in the field.

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

[2] This paper was prepared in the summer of 1940, when one of the writers was called to another post. It was held in the hope that the results of 1941 would yield conclusive evidence regarding insect transmission and provide some quantitative data on the influence of the disease on fruitfulness of the trees. The leaf symptom expression of the disease in Wisconsin was so poor in 1941 that results from the transmission experiments could not be taken in that season. The paper, with minor revision, is therefore being published,

Chlorotic areas of lighter than normal green appear on any part of the leaf lamina. They are very variable in size and shape, sometimes showing in this early stage a more or less definite ring-spot effect. If the leaves are not abscised at an early stage of symptom expression, the chlorotic areas become progressively paler green and then progressively yellow to the shade

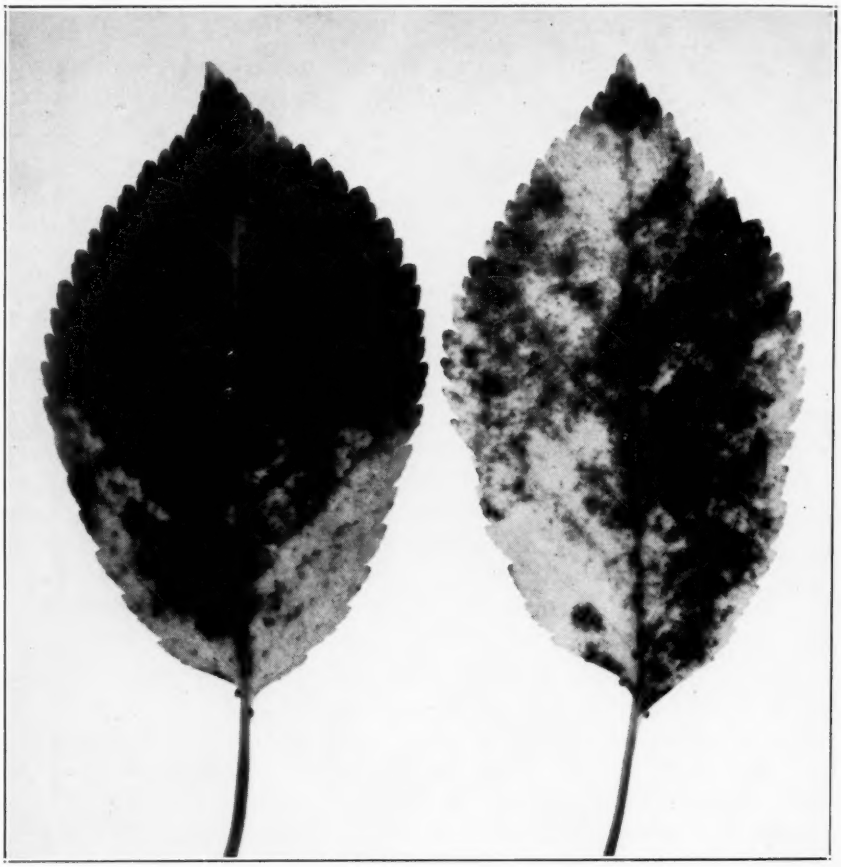

FIG. 1. Montmorency leaves showing symptoms of yellows. Photographed June 29, 1939.

characteristic of yellows leaves in the more advanced stages of symptom development. The range of yellow color is chiefly between the shades of Martius and Jasmine, as shown in the dictionary of color by Maerz and Paul (16). The affected leaves then exhibit a very conspicuous mottling of yellow and green (Fig. 1), and if they persist long enough on the tree, some of them may become entirely yellow. The green color may persist longest near the midrib and the main veins. Sometimes, however, this rela-

and it is contemplated that the results of work after 1940 will be treated in later publications.

Acknowledgments are made to J. D. Moore for assistance in the work done in 1940 and 1941; to Eugene Herrling for the photographic work.

tion does not obtain. In some cases the green fades and the yellow comes in rather uniformly over a large part of the leaf. Small islands of darker green may be left scattered in such areas. There is usually no necrosis, except occasionally at the tip of the leaf. These mottled or yellow leaves and some that are still green are soon abscised.

The leaf symptoms usually begin to appear about 3 or 4 weeks after petal-fall. A major wave of defoliation ordinarily begins in the Door County district in late June or early July and continues for about 2 or 3 weeks. A few affected leaves or minor waves of defoliation may be observed for some weeks later. The defoliation typically begins with the older leaves, commonly including the scale leaves, and extends towards the younger. Often, however, a leaf at or near the apex of a terminal shoot will show the symptoms in the latter part of the season, while the older leaves that remain on the shoot do not. The defoliation usually includes about 1 to 50 per cent of the leaves. Some of the leaves of diseased trees may be larger than normal, and the foliage of badly diseased trees, when viewed from a distance of about 10 to 50 feet, tends to appear lighter green than normal.

The disease does not kill the trees outright, but progressively and at variable rates over a period of years it impairs their health and fruitfulness, and probably tends to shorten their lives.

A fully authenticated description of the progressive development of the disease must await the results of extensive studies in which many cases are followed, with appropriate experimentation, throughout their complete histories. The following statements, which are subject to revision when more complete information is available, represent the writers' present judgment regarding certain aspects of the development of the disease as they have seen it in Wisconsin orchards.

The disease does not commonly seem seriously to impair the fruitfulness of the tree in the first year of symptom expression. Usually a few yellow leaves appear in the first year the disease is observed. By the second or third year defoliation may be severe and the crop much impaired. The rate of progressive development of the disease varies much with factors not yet

TABLE 1.—*Relation of cherry yellows to yield, Montmorency variety, Sturgeon Bay, Wis., 1941*

Year tree first mapped as diseased	Pairs[a] of trees	Average yield per diseased tree	Average yield per healthy tree	Indicated average reduction in yield due to virus	
	Number	*Pounds*	*Pounds*	*Pounds*	*Per cent*
1936	18	81.3	139.1	57.8	41.6
1937	6	71.0	125.8	54.8	43.6
1938	8	129.6	157.9	28.3	17.9
1939	14	107.9	129.6	21.7	16.7
1940	4	131.0	123.8	.....	.....

[a] Diseased trees were chosen from a survey map of the orchard without observation of the trees, and each was paired with an adjacent healthy tree selected at random from this map.

clearly understood. Among possible factors governing this rate of development are virus strains or combinations, variations in the hereditary resistance of host strains or varieties, and the play of varying environmental conditions.

Limited evidence of a progressive effect of the disease on fruitfulness of the trees is shown in data taken in 1941 (Table 1). There is no evidence of

FIG. 2. Fruiting branches from the more vigorous growth of diseased (left) and healthy (right) Montmorency trees. The diseased tree is known to have shown symptoms of yellows since 1936. Strings and arrows mark the annual linear growth. The scale shown is in inches. Photographed July 19, 1941. There was very little expression of the leaf symptoms of yellows in Wisconsin in 1941.

reduction in yield of the trees on which the symptoms of the disease were first observed in 1940. The reduction appears to have been moderate on trees known to have shown symptoms for 2 or 3 years and severe on trees known to have shown symptoms for 4 years or more. These statements, it should be noted, are based on averages from comparatively small numbers

of trees. It is planned that the detailed data of table 1, with statistical treatment, will be reported in a later publication with further data from the same and other trees. More extensive data on the comparative yield of diseased and healthy trees, without record of the length of time the diseased trees had shown symptoms, are given by Rasmussen and Cation (18). The average yield of the diseased trees reported by these investigators was approximately 47 per cent that of the healthy trees.

A detailed account of the effects of the disease on the growth and fruitfulness of the tree must await the results of further work. It may be said now that the spur system of the diseased tree tends progressively to become

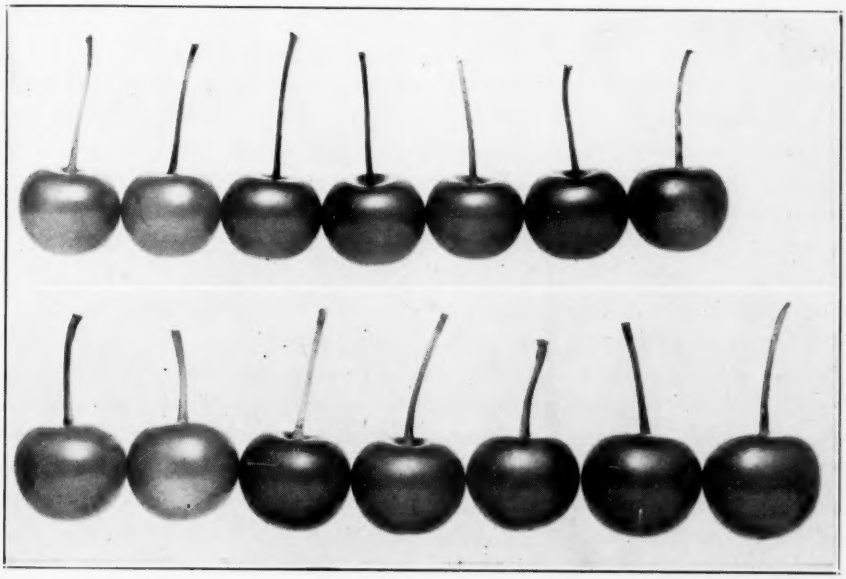

FIG. 3. Montmorency fruits from healthy (top row) and diseased (bottom row) trees.

much reduced over a period of years (*cf.* 17), in comparison with that of a healthy tree (Fig. 2).

The fruits from diseased trees are of good quality and free from any external or internal necrosis or bumpiness. They tend to be larger (often as much as 20 or 30 per cent) than average-size fruits from healthy trees (Fig. 3). Samples of 500 fruits, 10 fruits taken at random from each of 50 diseased trees, and similar samples from each of 50 adjacent healthy trees were collected on 2 dates in each of the years 1936–1939, inclusive. The average weight of the samples from the diseased trees was greater than from healthy trees in the stated years by the following percentages: 1936, 16; 1937, 32; 1938, 17; and 1939, 30. It is probable that the large size of the fruit is correlated in some degree with the sparseness of the crop (*cf.* 11). Brix tests in 1937 showed that sap of fruits from diseased and healthy trees, respectively, had about the same sugar content. The fruit symptoms of this

disease are in marked contrast to those described by Atanasoff (1) and Christoff (2) for mosaic of stone fruits in Europe and those of the pink fruit disease of sour cherry reported by Heald, Jones, and Huber (7) and Reeves, Huber, and Baur (20).

Diseased trees tend to bloom about 2 days later than healthy ones. The petals on some of their blossoms may have pink lines or splotches. The blossom buds of diseased trees have at times seemed to be more subject to winter injury or frost injury than those of healthy trees.

Symptoms on Montmorency and Early Richmond, the chief sour cherry varieties dealt with in this investigation, have seemed indistinguishable.

It is recognized that some of the symptoms described above may be atributable to a virus complex rather than a single virus, and that symptoms in addition to those described may be attributable to the yellows virus. In the latter regard it is noteworthy that, in greenhouse experiments that will be reported in a later paper, necrotic ring spot (9, p. 62–63) has commonly developed after chip budding from sour-cherry-yellows trees to presumably healthy sour cherry trees. A like result was reported earlier in correspondence with the writers by E. M. Hildebrand, who had budded in the greenhouse from yellows twigs procured from them. In extensive experiments in the greenhouse at Wisconsin both necrotic ring spot and a chlorotic ring spot have commonly appeared after chip budding from sour-cherry-yellows trees to presumably healthy sour-cherry trees and several other *Prunus* species. The necrotic ring spot is very prevalent in the orchards from which the yellows twigs were taken, occurring both on trees that have shown yellows symptoms and those on which yellows has not been observed. The chlorotic ring spot has been observed less frequently in the orchard but has commonly occurred in the greenhouse experiments on the same trees affected by necrotic ring spot. A rosette condition also has been encountered on sour cherry, in both orchard and greenhouse. The relations of yellows, necrotic ring spot, chlorotic ring spot, and rosette have not yet been satisfactorily clarified.

EXPERIMENTS ON THE CAUSE OF THE DISEASE

Microscopic examinations and other laboratory studies of the diseased tissues showed no evidence that a fungus or bacterium has a causal relation to the disease.

Various fertilizer treatments were started early in the course of this investigation in order to determine whether the "yellow leaf" condition was the result of a deficiency of some necessary element or whether addition of some element or elements might affect the course of the disease or the expression of symptoms. Ferrous sulphate, zinc sulphate, manganese sulphate, borax, ammonium sulphate, potassium chloride, and calcium superphosphate were used alone and in various combinations in relatively heavy applications to the soil under diseased and healthy trees in the fall of 1937 and the spring of 1939. Injections in the trunks of diseased trees were made in 1937 with

solutions of each ferrous sulphate, manganese sulphate, and zinc sulphate. Several diseased trees were severely pruned in 1938. Visual examinations of the experimental trees in 1938, 1939, and 1940 showed that none of the diseased trees had been cured. Additional experiments with fertilizing, mulching, and pruning were started in 1940 to discover further evidence regarding the extent to which the course of development of the disease and yield of the affected trees might be modified.

Transmission from Sour Cherry to Sour Cherry by Budding

The varieties used were Montmorency and Early Richmond. The results from these were indistinguishable and are, therefore, not listed separately.

Experiments of 1937–1938. Preliminary experiments were initiated in 1937 to determine whether the disease is bud-transmissible. The results in 1938, though inconclusive, strongly suggested that the disease had been transmitted.

Experiments of 1938–1939. In 1938, reciprocal budding experiments with diseased and healthy trees were started in 3 orchards. The budding treatments, number of trees used, and results in 1939 are shown in table 2.

TABLE 2.—*Summary of data on transmission of cherry yellows by budding, Sturgeon Bay, Wis., 1938–1939*

Budding of trees in 1938		Results in 1939				
Treatment	Treated or control trees	Shoots developed from inserted buds		Condition of trees		
		Healthy	Diseased	Healthy	Diseased	Doubtful
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
Buds from diseased trees[a] inserted in healthy trees	24	0	3	0	20	4[b]
Buds from healthy trees inserted in diseased trees	8	0	4	0	8	0
Controls: healthy trees not budded	13	...	...	13	0	0
Control: buds from healthy trees inserted in healthy tree	1	0	0	1	0	0

[a] Buds were inserted from 10 diseased trees, and the disease was transmitted from each of these trees.

[b] All these trees showed typical leaf symptoms of the disease in 1940.

The details are omitted, except for the chief experiment, which is briefly described as follows:

Twenty-eight 3-year-old Montmorency trees planted 6 feet apart in a row were used. Symptoms of the disease appeared on 1 of these trees in 1936, before the experiment was started, and each year thereafter.

One or more buds from diseased trees were inserted in each of 15 apparently healthy[3] trees in late July and early August, 1938. None of the buds

[3] Trees are hereinafter referred to as healthy when they showed no symptoms of the disease at the time of observation.

from diseased trees put out shoots, although union of tissues between the inserted bud-piece and the budded tree occurred in many cases. In 1939, 11 of these 15 budded trees showed definite leaf symptoms of the disease (Fig. 4). These leaf symptoms were more pronounced on the branches close to the place of bud insertion; however, some diseased leaves were found on all branches of the trees. Doubtful symptoms occurred on the remaining 4 budded trees. In each of these cases it was doubtful whether there had been union of tissues of bud-piece and branch. All of these 4 trees, without further treatment, showed typical symptoms in 1940.

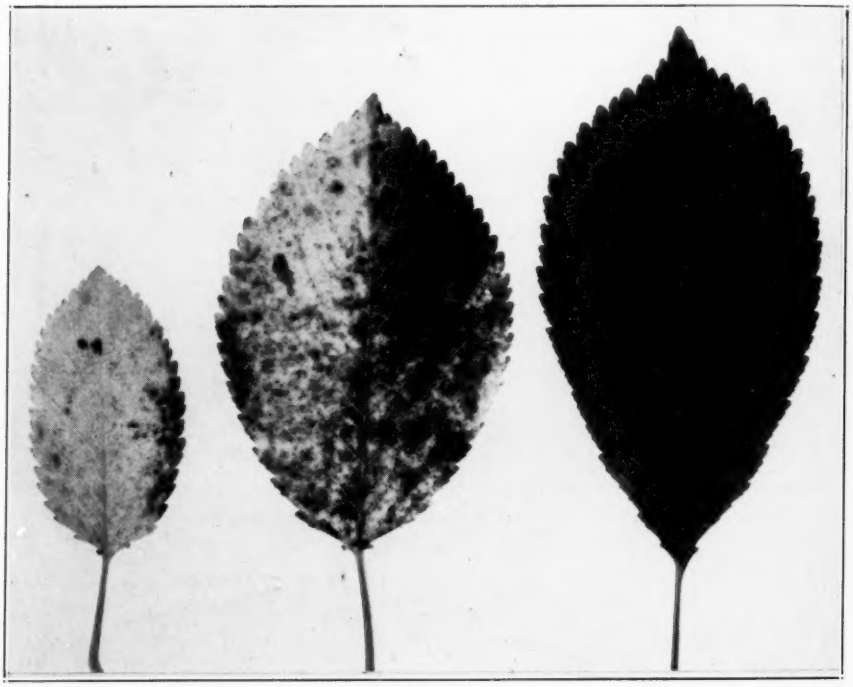

FIG. 4. Symptoms of yellows on leaves taken in serial order from the base of a shoot on a Montmorency tree to which the disease had been transmitted by budding in 1938. Only the 2 basal leaves of this shoot showed chlorosis when photographed on June 28, 1939. Two necrotic lesions, incited by *Coccomyces hiemalis* Hig., are seen on the first and third leaves.

A bud from a healthy tree was inserted in the tree (mentioned above) on which the disease had appeared in 1936. In 1939, the shoot from the inserted bud bore leaves showing the typical symptoms of the disease.

Twelve trees were left unbudded for controls. None of these showed symptoms of the disease in 1939.

Experiments of 1939–1940. The results of the budding experiments of 1939 are summarized in table 3. The details are omitted, except for the chief experiment, which is briefly described as follows:

Sixty-nine young Montmorency trees planted in 1939 at 2-foot intervals in rows 20 feet apart were used in the experiments on transmission of the disease by budding and by leaf hoppers. One of these trees showed the symptoms of the disease in 1939 before the experiments were started, and 2 others were doubtful.

Buds from diseased Montmorency and Early Richmond trees were inserted in 27 healthy trees and 1 that was doubtful. Twenty-six of these trees showed the characteristic leaf symptoms in 1940, and 2 were doubtful. In all cases in which union was observed between the tissues of bud-piece and

TABLE 3.—*Summary of data on transmission of cherry yellows by budding, Sturgeon Bay, Wis., 1939–1940*

Budding of trees in 1939		Results in 1940				
Treatment	Treated or control trees	Shoots developed from inserted buds		Condition of trees		
		Healthy	Diseased	Healthy	Diseased	Doubtful
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
Buds from diseased[a] trees inserted in healthy trees	52	0	1	6	40	6
Buds from healthy trees inserted in diseased trees	6	0	3	0	6	0
Controls: healthy trees not budded	32	...	...	26	0	6[b]
Controls: buds from healthy trees inserted in healthy trees	18	17	0	16	2	0

[a] Buds were inserted from 17 diseased trees and the disease was transmitted from each of these trees.

[b] Observations were complicated by occurrence of leaf spot.

tree the disease was transmitted. One of the diseased buds put forth a shoot, which was diseased.

A bud from a healthy tree was inserted in the tree that was diseased when the experiment was begun. The bud put out a shoot that showed the typical leaf symptoms in 1940.

Buds from healthy trees were inserted in 2 healthy trees. One bud put out a shoot, which was healthy. Both trees were healthy in 1940.

Twenty-two healthy trees were left unbudded as controls. An outbreak of leaf spot complicated the observation of results in 1940. Sixteen of these control trees showed no evidence of the symptoms of the virus disease. Six were noted as doubtful but were thought much more likely to be free of the disease than to have it.

It is noteworthy that the experiments summarized in table 3 included transmission of the disease from 8 trees to which it had been transmitted by budding in 1938. In 1939, buds from such trees were inserted in 22 healthy trees, all of which showed typical symptoms of the disease in 1940.

Discussion. The foregoing experiments show that the disease on sour cherry is readily transmissible by budding. Diseased buds inserted in healthy trees usually failed to put out shoots. This was doubtless due in large measure to the fact that blossom buds were much more prevalent than leaf buds on the bud wood used. However, some union of tissues of bud-piece and the budded tree commonly occurred, and in all such cases observed the disease was transmitted. Transmission of the disease occurred in some cases in which it was uncertain whether there had been such union of tissues.

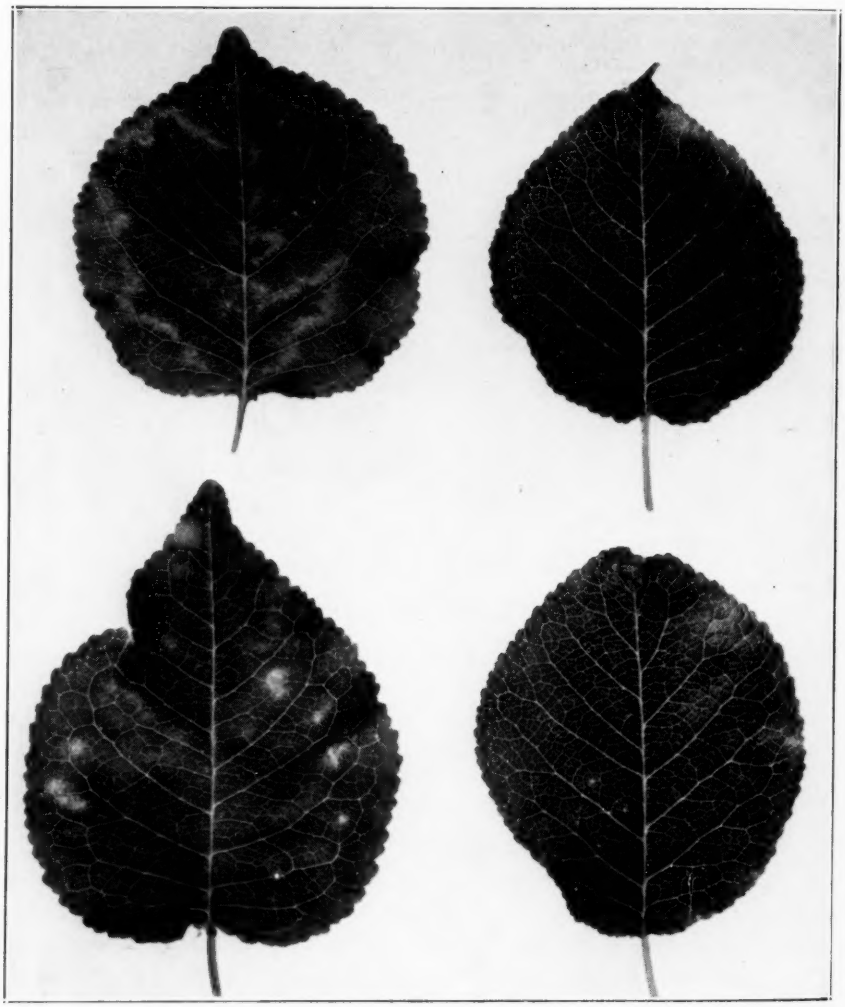

FIG. 5. Leaf symptoms on mahaleb to which the disease had been transmitted by budding from sour cherry. Left, diseased leaves from budded trees. Right, healthy leaves from controls.

Conclusions

From the lack of association of a bacterium or a fungus with the disease, the negative results from treatments with minor elements or fertilizers, the

results of the budding experiments, and the type of symptoms, it is concluded that the inciting cause of the disease is a virus or viruses.

PRELIMINARY EXPERIMENTS ON HOST RANGE

When, in the summer of 1939, it became clearly apparent that the disease is bud-transmissible and evidently incited by a virus or viruses, preliminary experiments on host range were initiated. Special interest attached to the 2

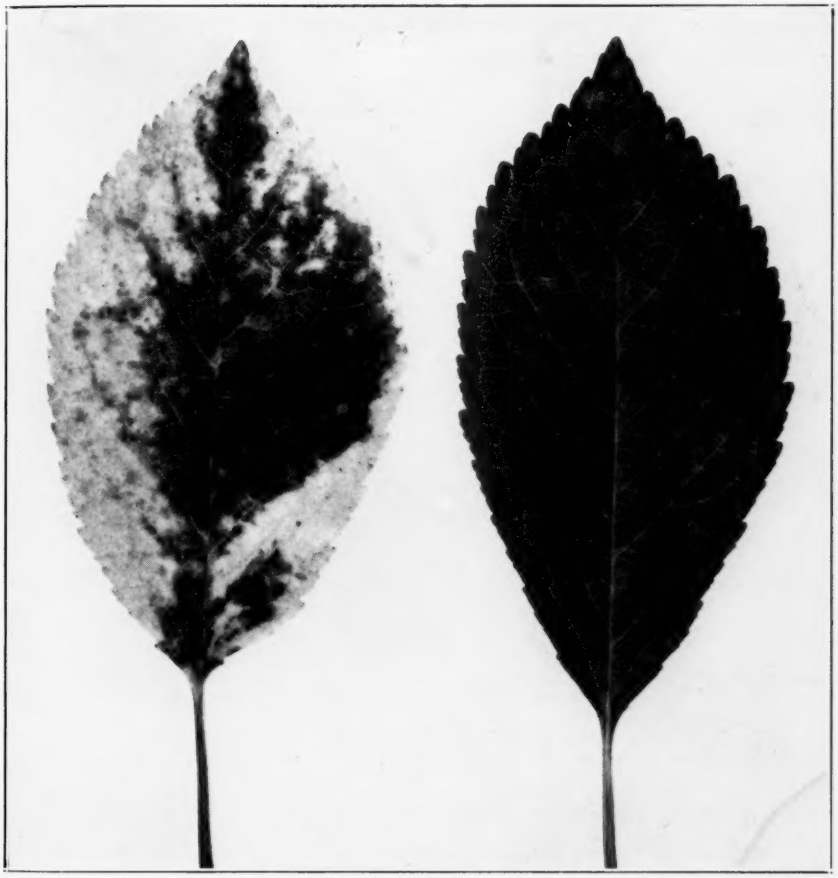

FIG. 6. Leaves from a large Montmorency tree to which the disease had been transmitted by budding from a mahaleb that had been budded from diseased sour cherry. Left, leaf taken near place of budding, showing typical symptoms. Right, leaf from opposite side of the tree, showing no symptoms. (The lighter areas represent spray residue.)

stocks on which sour cherry is commonly propagated, mahaleb (*Prunus mahaleb* L.) and mazzard (*P. avium* L.), and to other wild or cultivated species of *Prunus* that occur in the Door County section. It was necessary to use such plants as were available. The data thus far obtained are in part fragmentary, but in view of the importance of the disease it seems desirable

to report them briefly, rather than to await the results of more extensive studies.

Prunus mahaleb. In 1937, before transmissibility of the disease had been established, buds from diseased sour cherry were inserted in 5 healthy mahaleb sprouts. Two of these sprouts grew from the stocks of healthy sour cherry trees, 3 from the stocks of sour cherry trees that had died. In 1938, 1939, and 1940, chlorotic areas developed in some leaves of all of these budded sprouts (Fig. 5). Several similar untreated plants did not develop such symptoms. Similar symptoms have been observed on leaves of mahaleb sprouts from the stocks of diseased sour cherry trees.

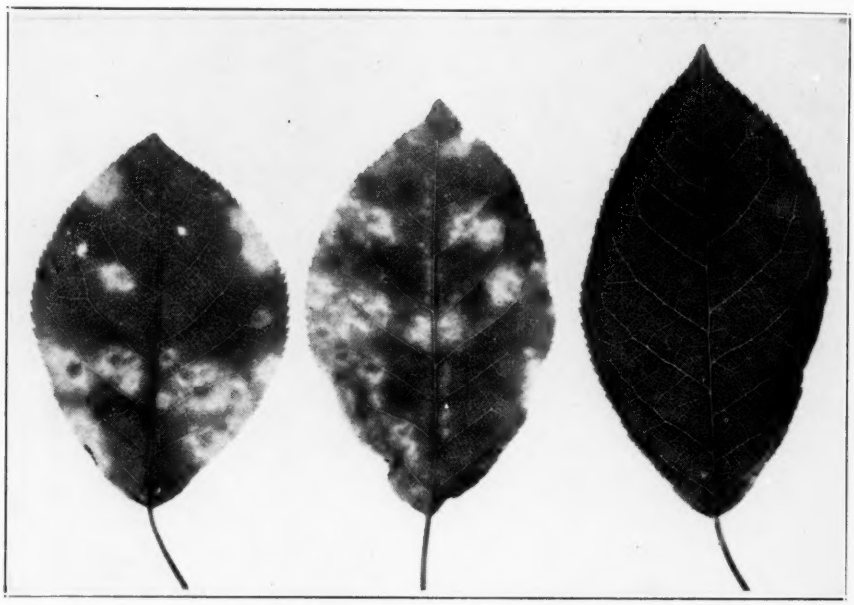

FIG. 7. Leaf symptoms on *P. virginiana* to which the disease had been transmitted by budding from sour cherry. A healthy leaf from a control tree is shown at the right.

Buds from the infected mahaleb sprouts were inserted in 2 healthy sour cherry trees in 1939. In 1940, the branches in which the buds were placed showed typical leaf symptoms of yellows, while the more remote branches showed no symptoms (Fig. 6). Several trees, left as unbudded controls, remained healthy.

Prunus avium. In 1939, buds from mazzard and 2 unknown cultivated varieties of sweet cherry were inserted in both healthy and diseased sour cherry trees. Six shoots grew from the buds placed in healthy trees and 7 from those placed in diseased trees. No definite symptoms of the disease appeared on any of these shoots in 1940.

Prunus virginiana L. In 1939, diseased sour cherry buds were inserted in several healthy chokecherry sprouts at one end of a large group. The re-

maining sprouts were not budded. Beginning in early June of 1940, leaves of all the budded sprouts showed abundant chlorotic ring spotting (Fig. 7). The affected leaves remained on the trees throughout the summer and did not develop the red color characteristic of chokecherry leaves affected by the X disease. The leaves of the control sprouts remained healthy. Similar ring spotting of natural occurrence on chokecherry (Fig. 8) was observed in 4 widely separated areas on the Door County Peninsula in 1940.

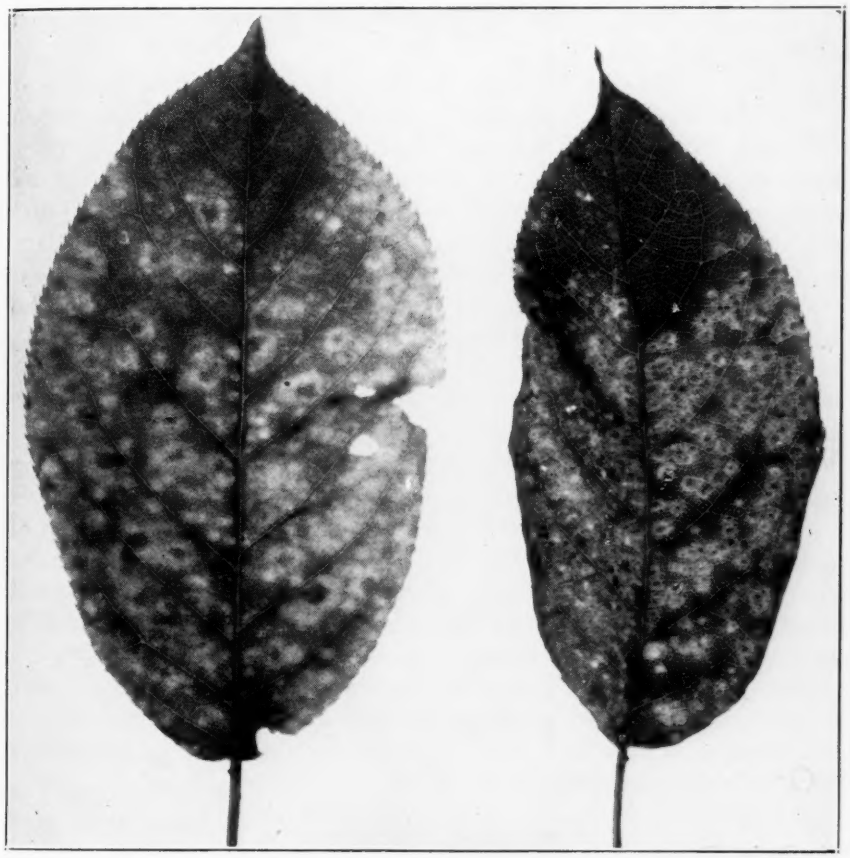

FIG. 8. Naturally occurring symptoms on leaves of a tree of *P. virginiana* located near a sour cherry orchard in which yellows was prevalent.

Prunus pennsylvanica L. Buds from diseased sour cherry trees were inserted in six healthy pin cherry sprouts in 1939. Union of tissues occurred on 2 of these. Chlorosis began to appear in the leaves of these 2 trees in June, 1940. Early in July some of the basal leaves dropped. The chlorotic areas on these leaves were at first light green, later yellow or pink. The leaves that remained on the trees showed light green chlorotic areas. The other 4 budded sprouts, and 6 similar unbudded ones, did not develop chlorosis. Similar chlorotic symptoms of natural occurrence were observed

on 1 pin cherry tree adjacent to a sour cherry orchard in which diseased trees were prevalent.

Prunus serotina Ehrh. Buds from diseased sour cherry were inserted in 2 healthy black cherry sprouts, and several similar sprouts were left unbudded. Union of tissues of bud-piece and sprout occurred in 1 case. In 1940, the basal leaves of this sprout showed light green chlorotic areas. These leaves were lighter green than normal and dropped in late June or early July. The unbudded sprouts showed no chlorosis. One black cherry tree showed naturally occurring symptoms similar to the chlorosis of the budded black cherry.

Discussion. The evidence that has been presented shows conclusively that the yellows disease is bud-transmissible from *P. cerasus* to *P. mahaleb* and back to *P. cerasus*. It is not yet certain whether the chlorotic symptoms incited on *P. mahaleb* are attributable to the yellows virus or to some associated virus or a virus complex. The data strongly suggest that the yellows virus or some associated virus or virus complex is bud-transmissible from sour cherry to *P. virginiana, P. pennsylvanica,* and *P. serotina.* The results with *P. avium* were negative, but subsequent experiments, which will be reported in a later paper, have shown that this species may carry the yellows virus.

In a recent paper Rasmussen and Cation (19) report transmitting cherry yellows from cherry to peach, peach to peach, and peach to cherry. They state that 3 strains of cherry yellows virus were differentiated by their reactions on peach and mahaleb cherry. Hildebrand (8)[4] reports that cherry yellows indexes readily on peach seedings, symptoms appearing in the greenhouse within 3 weeks after budding. Results similar to Hildebrand's have been obtained in greenhouse experiments now in progress with budded peach stock at Wisconsin. As has been noted by Hildebrand, Berkeley, and Cation (9), similar symptoms appear on peach trees affected by the severe ring spot virosis described by Cochran and Hutchins (5). Thomas and Rawlins (22) report a leaf mottle of sour cherry. Further work will be required to clarify the relationships of cherry yellows to these diseases and other viroses on stone fruits (*cf.* 9).

SPREAD OF THE DISEASE IN THE ORCHARD

Records of Incidence of the Disease

A record was taken annually from 1936 to 1940, inclusive, on each tree in 5 orchards in the vicinity of Sturgeon Bay, containing a total of 2,593 trees (Montmorency and Early Richmond, 11 to 29 years old in 1940). The observations were made during the major wave of defoliation. Each tree was classified as healthy, doubtfully or slightly diseased, moderately diseased, or severely diseased. Naturally, the classification of the stage of the disease varied somewhat with the time of observation and with the seasonal conditions.

[4] A fuller account of this work has appeared since the present paper was completed. (Hildebrand, E. M. Indexing cherry yellows on peach. Phytopath. 32: 712–719. 1942.)

TABLE 4.—*Rate of spread of cherry yellows in 5 orchards, Sturgeon Bay, Wis., 1936–1940*

Orchard No.	Variety	Age of trees in 1940	Trees observed	Trees moderately or severely diseased in stated years:				
				1936	1937	1938	1939[a]	1940
		Years	*Number*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	Montmorency	16	1250	7	7	10	16	18
2	Montmorency	15	207	7	11	26	26	33
3	Montmorency	11	372	1	1	5	8	9
4	Montmorency	19	240	0	1	4	7	7
5	Montmorency	29	220	2	4	5	8	8
2	Early Richmond	15	134	7	9	12	18	36
5	Early Richmond	29	170	0	1	1	5	6
	Total and weighted Avs.	.....	2593	4.6	5.8	7.4	13.2	16.6
	Av. increase over preceding year	.....	.....	.....	1.2	1.6	5.8	3.4

[a] In 1939 and 1940, the "moderate" class included some trees that would have been classed as "slight" in previous years.

The data, which are summarized in table 4, definitely establish the fact that the disease is spreading in the orchards. In 1936, 4.6 per cent of the trees in the 5 orchards were moderately or severely diseased, and in 1940, 16.6 per cent of the trees were thus diseased. The rate of spread varies widely with different orchards. The average yearly increase in incidence of the moderately and the severely diseased trees in the 5 orchards on which the records were taken was 3 per cent of the total number of trees.

The spread of the disease in a representative portion of orchard 1 of table 4 in each year from 1936 to 1940 is shown in figure 9. The percentage of trees moderately or severely diseased increased from 8 in 1936 to 22 in 1940. The increase varied from year to year, the average annual increase being 3.5 per cent of the total number of trees.

–	–	–	–	–	6	–	–	9	–	–	8	–	–	0	–	–	–	–	6
9	9	–	–	9	–	–	–	–	–	6	–	–	9	–	–	–	8	–	6
–	8	–	–	0	–	–	–	0	–	–	–	–	–	–	–		6	–	–
–	–	–	–	–	–	–	–	0	–	–	–	–	–	–	–	–	–	8	–
6	–	8	–	–	–	–	–	–	–	–	–	–	–	–	–		–	–	6
6	6	–	–	–	–	–	–	8	–	–	6	–	–	–	6	–	–	–	0
–	–	–		–	–	–	–	–	–	–	6	0	–	–	6	0	–	7	–
–	–	–	–	–	–	–	–	6	–	–	0	–	–	–	–	9	–	–	–
0	–	0	–	–		–	–	–	–	–	8	–	–	–	–	–	6	–	6
0	–	7		6	6	6	–	–	–	8	–	–	–	–	9	–	–	–	–
6	8	0	8	8	–	–	–	–	–	–	–		–	–	–	–	–	8	–
–	–	–	–	–	8	–	–	–	8	–	–	–	–	–	–	0	–		–
8	–	–	–	–	–	–	6	–	–	–	–	–	–	6	–	–	–	–	6
	–	–	–	9	–	–	–	–	8	–	–	–		–	–	8	–	–	–
–	–	7	–	–	–	–	–	–	6	–	0	–	–	6	–	0	–	–	6
	–	–	–	–	–	–	–	9	–	–	–	–	–	–	–	–	–	–	–

NUMBER OF TREES OBSERVED, 310

PERCENTAGE OF TREES DEFINITELY DISEASED:

1936, 8 1937, 9 1938, 14 1939, 17 1940, 22

FIG. 9. Incidence[5] of cherry yellows in a portion of orchard 1 (Table 4), Montmorency variety, Sturgeon Bay, Wis., 1936–1940.

In 1940, records of the incidence of diseased trees in 18 orchards containing 6,588 trees from 1 to 30 years old were taken in the Door County peninsular district in the manner described above. The data are summarized in table 5. The percentage of the moderately and the severely

[5] Explanation of symbols: – = healthy trees; 6 = trees on which definite symptoms of the disease were observed in 1936, when the survey was initiated; 7, 8, 9, 0 = trees on which definite symptoms were first observed in 1937, 1938, 1939, and 1940, respectively.

TABLE 5.—*Incidence of cherry yellows in 18 orchards, Door County, Wis., 1940*

Orchard No.	Variety	Age of trees in 1940	Trees observed	Trees in different stages of the disease in 1940			
				Slight	Moderate	Severe	Total of moderate and severe
		Years	*Number*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	Montmorency	1	240	1.6	0.4	0.0	0.4
2	Early Richmond	2	82	0.0	22.0	2.4	24.4
3	Montmorency	3	40	12.5	30.0	7.5	37.5
4	Montmorency	1 to 8	680	0.9	0.6	1.5	2.1
5	Montmorency	8	520	2.1	1.0	0.6	1.6
6	Montmorency	8	260	16.2	17.3	7.0	24.3
7	Montmorency	8	333	3.6	3.3	0.6	3.9
8	Montmorency and Early Richmond	9	807	1.1	1.2	2.0	3.2
9	Early Richmond	10	122	0.8	1.6	7.4	9.0
10	Montmorency	11	360	2.8	5.8	1.4	7.2
11	Montmorency and Early Richmond	15	344	9.0	24.7	13.9	38.6
12	Montmorency	16	434	1.8	2.0	8.3	10.3
13	Montmorency	16	1250	3.7	9.7	7.4	17.1
14	Montmorency and Early Richmond	10 to 30	161	0.6	3.1	5.0	8.1
15	Montmorency and Early Richmond	20	354	1.1	1.1	7.6	8.7
16	Montmorency and Early Richmond	10 to 30	156	1.9	6.4	23.1	29.5
17	Montmorency and Early Richmond	10 to 30	125	0.6	0.6	5.6	6.2
18	Montmorency and Early Richmond	29	320	1.6	3.8	2.8	6.6
	Total and weighted Avs.	.....	6588	3.0	5.7	5.0	10.7

diseased trees in the several orchards varied from 0.4 to 38.6, the weighted average for all orchards being 10.7 per cent. There is very little evidence of correlation between the age of the orchard and the percentage of diseased trees. However, there is probably a correlation between the number of trees diseased when received from the nursery and the number of diseased trees in the orchard. For instance, the nursery stock used for planting orchard 1 must have been nearly free from the disease, while that used for planting orchards 2 and 3 evidently included a considerable number of diseased trees. Furthermore, there is evidence that demand for large-fruited strains of sour cherry has led to some selection of buds from diseased trees. Orchards 6 and 7 are adjacent blocks, planted at the same time and in the same manner. The trees in orchard 6 were specially propagated as large-fruited strains of Montmorency. Those of orchard 7 were ordinary Montmorency. It is noteworthy that at the time of observation the percentage of diseased trees in the former orchard was 6 times as great as in the latter.

The rate of incidence of the disease appears to be about the same on Montmorency and Early Richmond; and trees of all ages seem susceptible (the disease was transmitted by budding to trees ranging from 1 to 29 years old).

These data show that the disease is steadily spreading in the orchards but indicate that the rate of spread is less rapid than is the case with some other virus diseases of stone fruits.

Preliminary Experiments on Insect Transmission[6]

In August, 1939, a preliminary experiment was initiated to seek evidence on whether leaf hoppers (Cicadellidae) transmit the disease. Leaf hoppers of a mixed collection, predominantly of 3 species, taken from apple trees, were caged from 1 to several days on diseased Montmorency cherry trees, then transferred for 8- to 15-day periods to caged branches of 1-year-old Montmorency trees in the planting used in 1939 for experiments on transmission of the disease by budding. The same untreated trees that served as controls for the budding experiments were used as controls. In 1940, 4 of the 10 trees that had been subjected to infestation by leaf hoppers that had fed on diseased trees were definitely diseased, 4 showed doubtful results, and 2 showed no evidence of the symptoms. Of the 22 untreated controls, 16 were healthy, 6 were doubtful, and none were definitely diseased. As stated above, the occurrence of leaf spot on these trees complicated the readings. It is thought that the control trees recorded as doubtful are much more likely to have been free of the virus disease than to have had it.

Though confirmation and extension of this preliminary experimentation will be necessary before final conclusions are justified, the results give very strong evidence that the disease can be transmitted by leaf hoppers. Further experiments are in progress.

Preliminary experiments were initiated in 1939 to test the possibility that the black cherry aphid (*Myzus cerasi* Fab.) might transmit the disease. Aphids taken on July 3 from naturally infected sour cherry trees were placed on the leaves of 6 one-year-old Montmorency trees that were entirely enclosed in cheesecloth cages. Large colonies of aphids developed on leaves of 2 of these trees. Two weeks after the aphids had been placed on the leaves, the cages were removed and the trees sprayed with a nicotine sulphate solution to destroy the insects. No symptoms of the disease were observed in 1940 on these trees or on 10 similar untreated trees that served as controls.

In other experiments in 1939, black cherry aphids were transferred from diseased sour cherry trees to 23 healthy ones, most of which were 1 to 5 years old. There was little natural occurrence of the disease in the orchards in which the tests were made. Approximately 25 to 300 aphids were placed

[6] The work on insect transmission is being done in cooperation with John H. Lilly, Department of Economic Entomology, University of Wisconsin, and this preliminary statement is published with his approval.

on each tree, and no cages were used. In 1940, all of the 23 trees remained healthy, as did 10 untreated trees.

The results of these experiments, though not conclusive, give strong evidence that the disease is not transmitted by the black cherry aphid.

CONTROL MEASURES

Recommendation of a complete program for control of cherry yellows must await the results of further studies. However, the available information clearly indicates certain steps that should be taken promptly. Since spread of the disease through nursery stock is an important source for its establishment in the orchard, the production and use of disease-free nursery stock is of prime importance. Young orchard plantings should be systematically inspected and diseased trees promptly removed when symptoms appear. In older orchards in which comparatively few trees are diseased, it would seem advisable similarly to remove the diseased trees in order to lessen the chances for spread of the trouble. Recommendations regarding older orchards in which the disease is well established are withheld until a fuller knowledge of the disease can be obtained. In the meantime, growers are advised to mark and observe all trees that show symptoms of yellows.

SUMMARY

A destructive disease of sour cherry (*Prunus cerasus*), tentatively named cherry yellows, is incited by a virus or viruses. Affected trees tend to have relatively large leaves, some of which develop conspicuous chlorotic areas. The chlorotic leaves and some that are still green are abscised, a major wave of defoliation usually occurring about 3 or 4 weeks after petal-fall. Diseased trees probably live nearly as long as healthy ones but tend to have fewer spurs and sparse crops.

Reciprocal budding experiments between diseased and healthy sour cherry trees resulted in transmission of the disease in all cases in which union of tissues of bud-piece and budded tree was observed.

The virus was transmitted also by budding from *P. cerasus* to *P. mahaleb* and back to *P. cerasus*, though it is not certain whether the chlorotic symptoms incited on *P. hamaleb* are attributable to the yellows virus. Limited experiments on transmission to other *Prunus* species are reported.

Records of incidence of moderately or severely diseased trees, taken from 1936 to 1940, inclusive, in 5 orchards containing 2,593 trees, showed an average annual increase of 3 per cent. In 1940, the percentage of such diseased trees in 18 orchards, containing 6,588 trees, was 10.7. Montmorency and Early Richmond, the varieties dealt with in this work, appear to be equally susceptible to the disease.

Preliminary experiments gave strong though not conclusive evidence that the disease can be transmitted by leaf hoppers (Cicadellidae). Simi-

lar experiments with the black cherry aphid (*Myzus cerasi* Fab.) gave only negative results.

Further experiments on this disease and its control are in progress.

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PATHOGENICITY STUDIES WITH FUSARIA ISOLATED FROM TOBACCO, SWEET POTATO AND COTTON[1]

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INTRODUCTION

Fusarium wilt of tobacco (*Nicotiana tabacum* L.), commonly attributed to *Fusarium oxysporum* Schl. v. *nicotianae* Johnson (10), is a minor disease of flue-cured tobacco. It is of economic importance at present only in Columbus County, North Carolina, near Whiteville and Chadbourn, where it has been present since about 1931. The disease is becoming more serious as evidenced by spread during recent years in this and other areas. Fusarium wilt of tobacco also has been reported from Maryland, Ohio, Kentucky, Canada, South Africa, and Russia. The disease was attributed to *F. oxysporum nicotianae* in all reports, but, in South Africa, Doidge (7) also isolated in one instance, *F. bulbigenum* from all parts of the discolored wood from roots to petiole. Fusarium wilt of cotton (*Gossypium hirsutum* L.), commonly attributed to *F. vasinfectum* Atk., is a major disease, more or less universally present wherever this crop is grown. Fusarium wilt or stem rot of sweet potato (*Ipomea batatas* (L.) Lam.), commonly attributed to *F. bulbigenum* Cke. and Mass. v. *batatas* Wr. and *F. oxysporum* Schl. f. 2 Wr., is a major disease of this crop and is also more or less universally present. In the flue-cured tobacco belt, cotton, sweet potato, and tobacco are often grown in rotations on the same fields. The host range of Fusaria from these crops was originally considered different, hence their use in rotations was unlimited. However, the work of Armstrong (1, 2, 3) and others raised several questions about the indiscriminate growth of these crops on the same soil during successive years.

RELATION OF SWEET POTATO ROTATIONS TO OCCURRENCE OF FUSARIUM WILT IN FLUE-CURED TOBACCO

At Creedmoor, North Carolina, a few tobacco plants developed fusarium wilt when grown in a rotation experiment with Granville wilt (*Bacterium solanacearum* E.F.S.). The fusarium-infected plants were found in 1940 on a plot where sweet potato was grown in 1936 and 1937, tobacco in 1938 and sweet potato in 1939. No fusarium wilt developed on tobacco grown after redtop grass, crab grass, corn, native weeds, or tobacco. Isolations of *Fusarium* from tobacco were pathogenic to tobacco and sweet potato. This was the only known occurrence of fusarium wilt in the Creedmoor area.

[1] Cooperative Investigation by U. S. Department of Agriculture, N. C. Agricultural Experiment Station and the N. C. Department of Agriculture. Published with the approval of the Director, North Carolina Agricultural Experiment Station, as paper no. 146 of the Journal Series.

[2] The writers express appreciation for the cooperation of many persons who sent collections of plant material and cultures or allowed use of their unpublished observations.

At the McCullers Branch Station, McCullers, North Carolina, fusarium wilt of tobacco first appeared in 1938 on a plot planted in 1937 to sweet potato heavily inoculated with a sweet-potato isolate collected in New Jersey in 1925 by R. F. Poole.[3] A part of this plot was planted to tobacco in 1939, 1940, and 1941, and fusarium wilt was present every year, reaching a maximum of 50 per cent kill. Cotton, soybeans, and sweet potato have have also been grown on this plot in rotation with tobacco but only tobacco and sweet potato had fusarium wilt. In 1940, fusarium wilt of tobacco appeared in a crop-rotation experiment being conducted on the root-knot disease in a different area of the farm. This experiment was a randomized block design with 10 rotations in 4 blocks and the fusarium wilt was present on tobacco in all 4 plots previously planted to sweet potato, but was absent on tobacco grown after corn, cotton, peanuts, velvet beans, soybeans, crotalaria, native weeds, oats and native weeds, and bare fallow. The plots were enclosed with creosoted-pine boards to control surface water and all cultivation was done with well-cleaned hand tools. Therefore, it was unlikely that the disease was introduced by surface water or contaminated implements. A small amount of fusarium wilt had been observed on sweet potato whenever this crop was grown.

A few tobacco plants with fusarium wilt were observed in 1940 on a field planted in 1939 to sweet potato at the Pee Dee Experiment Station, Florence, South Carolina (reported by T. W. Graham[3]).

Similar observations were made at the Georgia Coastal Plain Experiment Station, Tifton, Georgia, by J. G. Gaines.[3] In a rotation experiment being conducted on the root knot disease, fusarium wilt of tobacco was observed on a plot where sweet potato was grown the previous year. No fusarium wilt developed on tobacco grown after cowpeas, cotton, corn, peanuts, velvet beans or native weeds on other plots of the experiment. The cotton was badly wilted during some years. In 1930, 1931, 1932, and 1933, cotton was killed 34 to 55 per cent but tobacco grown the succeeding years showed no wilt. Thus, fusarium wilt of flue-cured tobacco was associated with sweet potato rotations at 4 locations in 3 States. But no wilt was observed on tobacco grown after cotton at these locations.

OBSERVATIONS ON THE OCCURRENCE OF FUSARIUM WILT OF BURLEY TOBACCO

Fusarium wilt of Burley tobacco was reported by Armstrong (1) at 6 locations where there was no record of previous culture of this crop. The tobacco was grown on cotton farms and it was later demonstrated that Fusaria from cotton, okra, and coffee weed (*Cassia tora*) were pathogenic to Burley tobacco. Similar observations were made by E. E. Clayton,[3] who, in 1941, found fusarium-infected Burley in Northeastern Virginia, where, likewise, there was no record of previous tobacco culture. In addition to these observations, fusarium wilt has been generally found on Burley in plantings made for experimental purposes throughout the Southeastern

[3] Personal communications.

States. It appears that fusarium wilt of Burley was associated with rotations of cotton and perhaps other plants.

PATHOGENICITY TRIALS WITH FUSARIA ISOLATED FROM TOBACCO, SWEET POTATO AND COTTON

Methods

Several different methods of inoculation were tried: (1) Stem inoculation with bits of discolored woody tissue from diseased plants. (2) Soil inoculation with cultures grown on cornmeal-sand medium. (3) Soil inoculation with macerated diseased stems. (4) Root inoculation made by pouring diluted cultures in holes punched in the soil around potted plants. (5) Dipping the roots of plants, prior to transplanting, in a diluted culture. Some infection was obtained with methods 1, 2, 3, and 4, but the results were not consistent. Method 5, a modification of that described by Wellman (16), was finally adopted because of its simplicity and the fact that the results could be duplicated in repeated trials.

Fusaria were isolated by the writers from fresh specimens of wilted plants, designated by collection numbers in the tables, or cultures were obtained from other workers as indicated. Inoculum was prepared by diluting macerated agar-slant cultures or by diluting liquid cultures grown on the mineral-nutrient solution plus 1 per cent dextrose. Preliminary trials showed that dilutions of 1 part culture to 5 or 20 parts total volume made no difference in the end effect. The inoculation technique was to rinse the loosely-held soil from the roots, dip them in diluted inoculum and set the plants into steam-sterilized sand in pots or flats. Unwashed sand obtained from streams and roadside ditches was used. Inoculated plants were carefully and adequately protected from accidental contamination by other Fusaria, as illustrated by the fact that the noninoculated controls, approximately 40 in all, remained healthy. The plants were watered once daily with nutrient solution and with tap water as often as required. The nutrient solution was prepared by adding boron, manganese, and iron, in proper amount, to the stock solution used by McMurtrey (11).

Fusarium-susceptible strains of test plants were used for all inoculations. The Jamaica variety of flue-cured tobacco, Judy Pride variety of Burley tobacco, Porto Rico variety of sweet potato, and Half and Half or Cokers 100–1 varieties of cotton were grown. These agronomic varieties were employed because they are in general use by farmers in the Southeastern States and are representative of the fusarium-susceptible strains of their respective crops. Seedlings of tobacco and cotton were grown from surface-sterilized pure-bred seed. Cotton was transplanted with ease if taken up before the lateral roots became more than 1 or 2 inches long. Sprouts of sweet-potato roots or rooted cuttings of field-grown vines were used for inoculations on this plant.

In recording the results of inoculations, reliance was placed in the type of symptoms observed on the various plants following natural infection

under field conditions. In most cases this was possible because the plants made normal growth before wilt symptoms developed. Thus, pathogenicity on tobacco and sweet potato was characterized at first by yellowing of the leaves, followed later by wilting and in severe cases death. On cotton the first symptom was wilting of the green leaves. Extensive discoloration of the stele occurred almost simultaneously with wilting. The results on Burley tobacco, sweet potato, and cotton were clear-cut, that is, these symptoms were either present or absent and are shown in the tables as "+" and "−", respectively. On flue-cured tobacco, however, an intermediate reaction often was obtained, which was characterized by wilting of leaves while still green or by severe stunting. These symptoms have not been associated with fusarium wilt of tobacco under field conditions and were not considered typical pathogenic effects, but atypical symptoms resulting from heavy inoculations. Therefore, the intermediate reaction, indicated in the tables by "±," was largely ignored in interpreting the results. Some pathogenic effects, such as limited discoloration of the stele and slight stunting, followed nearly all inoculations classed as negative, but these were disregarded in recording the results.

Inoculation trials were conducted from August, 1940, through January, 1942, in the greenhouse at the Tobacco Branch Station, Oxford, North Carolina. Air temperatures varied from a maximum of 110° F. on bright days in mid-summer to a minimum of about 60° F. at night and on cloudy days in mid-winter. Results obtained under the higher temperatures of summer were duplicated many times under the lower temperatures of mid-winter, by allowing a longer period of time to elapse between inoculation and final note-taking. In summer a 15-day period was often adequate, but in winter 30 or 45 days were often required for the development of decisive results. The accuracy of the observations was repeatedly verified by growing the plants to maturity.

Inoculations with Fusaria from Tobacco

Fifty-three collections from various commercial types of tobacco from 5 States were tested and are considered representative of fusarium wilt of tobacco as it occurs in this country. The results of inoculations on flue-cured tobacco, Burley tobacco, sweet potato, and cotton are summarized in table 1. The collections are tabulated by type of tobacco and by State of origin for convenience in reviewing the results. Two different physiologic races were collected: (1) The race pathogenic on flue-cured tobacco, Burley tobacco, and sweet potato but nonpathogenic on cotton. (2) The race pathogenic on Burley tobacco and cotton and giving the intermediate reaction on flue-cured tobacco, in most cases, but nonpathogenic on sweet potato. In repeated trials all isolations were remarkably consistent except on flue-cured tobacco. There was a tendency for certain collections, during storage or following reisolation, to lose virulence for this test plant, even though they were originally collected, in some cases, from this type of tobacco.

TABLE 1.—*Results of inoculations with Fusaria isolated from tobacco*

Collection and year originally isolated	Type of tobacco	Collected at	Result of inoculations on							
			Flue-cured tobacco			Burley tobacco		Sweet potato		Cotton	
			+[a]	±	−	+	−	+	−	+	−
1; 1940	Flue-cured	Creedmoor, N. C.	5[b]	0	0	1	0	5	0	0	4
7; 1940	do.	Chadbourn, N. C.	17	0	0	2	0	17	0	0	16
8; 1940	do.	Whiteville, N. C.	2	0	0	1	0	2	0	0	1
9; 1940	do.	do.	3	0	0	1	0	3	0	0	3
10; 1940	do.	do.	1	0	1	1	0	2	0	0	1
11; 1940	do.	do.	1	0	1	1	0	2	0	0	2
12; 1940	do.	do.	3	0	0	1	0	3	0	0	3
13; 1940	do.	do.	1	0	1	1	0	2	0	0	2
14; 1940	do.	do.	1	0	1	1	0	2	0	0	2
15; 1940	do.	do.	1	0	1	1	0	2	0	0	2
19; 1940	do.	McCullers, N. C.	1	0	2	1	0	3	0	0	2
20; 1940	do.	do.	1	0	1	1	0	2	0	0	2
69; 1941	do.	Chadbourn, N. C.	1	0	0	−	−	1	0	0	1
92; 1941	do.	Whiteville, N. C.	2	0	0	1	0	2	0	0	3
99; 1941	do.	do.	2	0	0	1	0	2	0	0	2
2; 1940	do.	Florence, S. C.	3	0	0	1	0	3	0	0	3
4; 1940	do.	Tifton, Ga.	1	0	1	1	0	2	0	0	2
6; 1940	do.	do.	1	0	1	1	0	2	0	0	2
103; 1941	do.	do.	2	0	0	1	0	2	0	0	3
104; 1941	do.	do.	2	0	0	1	0	2	0	0	2
105; 1941	do.	do.	0	4	0	3	0	0	4	4	0
33; 1940	Maryland	Upper Marlboro, Md.	1	0	1	1	0	2	0	0	2
34; 1940	do.	Maryland	1	0	1	1	0	2	0	0	2
35; 1940	do.	do.	3	0	0	1	0	3	0	0	3
36; 1940	do.	do.	1	0	1	1	0	2	0	0	2
107; 1941	do.	do.	1	0	0	1	0	1	0	0	2
E. A. Walker's single spore cultures; 1939	do.	Charles Co., Md.	1	0	1	1	0	2	0	0	3
	do.	do.	1	0	0	1	0	1	0	0	1
	do.	do.	0	0	1	1	0	1	0	0	1
	do.	do.	1	0	0	1	0	1	0	0	1
130; 1941	do.	do.	1	0	0	1	0	1	0	0	1
E. M. Johnson's culture; 1937	do.	Florence. S. C.	0	1	1	2	0	0	2	2	0
G. M. Armstrong's culture	Burley	South Carolina	0	3	3	3	0	0	6	6	0
	do.	do.	0	2	1	1	0	0	3	3	0
108; 1941	do.	Lexington Ky.	1	0	0	−	−	1	0	0	2
E. M. Johnson's culture; 1937	do.	do.	2	0	0	1	0	2	0	0	3
Do.; 1937	do.	do.	2	0	0	1	0	2	0	0	2
Do.; 1937	do.	do.	2	0	0	1	0	2	0	0	2
Do.; 1937	do.	do.	1	0	0	1	0	1	0	0	1
Do.; 1937	do.	do.	0	1	0	1	0	0	1	1	0

TABLE 1.—*Concluded*

Collection and year originally isolated	Type of tobacco	Collected at	Result of inoculations on							
			Flue-cured tobacco			Burley tobacco		Sweet potato		Cotton	
			+[a]	±	−	+	−	+	−	+	−
E. M. Johnson's culture; 1935	Burley	Carter City, Ky.	2	0	0	1	0	2	0	0	2
Do.; 1935	do.	Middlesboro, Ky.	0	1	1	1	0	0	2	3	0
Do.; 1935	do.	Spurlington, Ky.	2	0	0	1	0	2	0	0	3
Do.; 1935	do.	Maysville, Ky.	3	0	0	1	0	3	0	0	3
Do.; 1937	do.	Logan Co., Ky.	0	2	0	1	0	0	2	2	0
Do.; 1937	do.	Todd Co., Ky.	0	2	0	1	0	0	2	3	0
Do.; 1937	do.	do.	0	0	1	1	0	0	1	1	0
Do.; 1937	do.	Jefferson Co., Ky.	1	0	0	1	0	1	0	0	1
Do.; 1937	do.	Christian Co., Ky.	1	0	0	1	0	1	0	0	1
Do.; 1933	do.	Mercer Co., Ky.	0	1	0	1	0	1	0	0	1
Do.; 1935	do.	Boone Co., Ky.	1	0	0	2	0	2	0	0	2
Do.; 1937	One-sucker dark	Logan Co., Ky.	0	1	1	1	0	0	2	2	0
Do.; 1937	do.	Todd Co., Ky.	0	1	1	2	0	0	2	2	0
Do.; 1937	do.	Simpson Co., Ky.	0	1	0	1	0	0	1	1	0
Do.; 1931	Dark-fire-cured	Graves Co., Ky.	1	1	1	3	0	3	0	0	4
Do.; 1937	do.	Simpson Co., Ky.	0	0	1	0	2	0	1	2	0
Do.; 1937	do.	Logan Co., Ky.	0	1	0	1	0	0	1	1	0

[a] Explanation of methods used in recording results: + means pathogenic, characterized by extensive discoloration of stele in combination with yellows and wilt on tobacco and sweet potato or wilt only on cotton. ± means intermediate reaction, characterized by severe stunting or slight wilt of green leaves. − means nonpathogenic.

[b] To obtain the total trials on each test plant, add the entries in each cell of the table.

Collection number 7 from Chadbourn, North Carolina, was the most virulent and this culture maintained virulence for flue-cured tobacco and sweet potato, without transfer, for 12 months.

In considering isolations from flue-cured tobacco, all the isolates except one (No. 105 from Tifton, Georgia) belong to the race pathogenic on sweet potatoes. Among isolates from Maryland tobacco, the race pathogenic on sweet potato also predominated, but one collection (Johnson's Culture; 1937) from the experimental plots at Florence, South Carolina, was pathogenic on cotton. From Burley, one-sucker, and dark-fire-cured tobaccos, the race pathogenic for tobacco and sweet potato and the race pathogenic for

Burley and cotton were obtained in approximately equal numbers. Armstrong's collection from Burley in South Carolina was pathogenic on cotton, as pointed out by that investigator (3).

Results of Inoculations with Fusaria from Sweet Potato

Twenty-two collections from sweet potato were tested, 19 from widely scattered localities in North Carolina, 2 from South Carolina and 1 from Georgia. The results of inoculation trials are given in table 2. All

TABLE 2.—*Results of inoculations with Fusaria isolated from sweet potato*

Collection and year originally isolated	Collected at	Results of inoculations on							
		Flue-cured tobacco			Burley tobacco		Sweet potato		Cotton	
		+[a]	±	−	+	−	+	−	+	−
41; 1940	McCullers, N. C.	1[b]	0	1	.....	.....	2	0	0	1
42; 1940	do.	0	1	0	1	0	2	0	0	1
43; 1940	Creedmoor, N. C.	0	1	1	1	0	2	0	0	1
51; 1940	do.	0	0	2	1	0	2	0	0	1
57; 1940	Oxford, N. C.	0	0	2	1	0	2	0	0	1
58; 1940	Creedmoor, N. C.	0	0	2	1	0	2	0	0	1
59; 1940	do.	0	0	3	1	0	3	0	0	2
60; 1940	Raleigh, N. C.	0	0	4	1	0	4	0	0	3
61; 1940	do.	1	5	7	1	0	13	0	0	13
62; 1940	do.	0	1	1	1	0	2	0	0	1
64; 1940	Oxford, N. C.	0	0	2	1	0	2	0	0	1
70; 1941	Whiteville, N. C.	1	0	1	1	0	2	0	0	2
80; 1941	do.	1	0	1	1	0	2	0	0	2
83; 1941	Kinston, N. C.	0	1	1	1	0	2	0	0	2
89; 1941	do.	1	0	1	1	0	2	0	0	2
91; 1941	Dover, N. C.	1	0	1	1	0	2	0	0	2
93; 1941	Whiteville, N. C.	2	0	0	1	0	2	0	0	2
94; 1941	Dover, N. C.	0	0	2	1	0	2	0	0	2
95; 1941	Clinton, N. C.	1	1	0	1	0	2	0	0	2
72; 1941	Florence, S. C.	0	1	1	1	0	2	0	0	2
73; 1941	Nichols, S. C.	0	2	0	1	0	2	0	0	2
102; 1941	Tifton, Ga.	0	0	2	1	0	2	0	0	2

[a] Explanation of methods used in recording results: + means pathogenic, characterized by extensive discoloration of stele in combination with yellows and wilt on tobacco and sweet potato or wilt only on cotton. ± means intermediate reaction, characterized by severe stunting or slight wilt of green leaves. − means nonpathogenic.

[b] To obtain the total trials on each test plant, add the entries in each cell of the table.

collections were consistently pathogenic on Burley tobacco and sweet potato but nonpathogenic on cotton. On flue-cured tobacco, 8 of the 22 collections were able to cause typical fusarium wilt symptoms in one or more trials. Collections from McCullers and Whiteville, North Carolina, were expected to be pathogenic for tobacco because fusarium wilt on flue-cured tobacco is generally present in the Whiteville area and to a limited extent at McCullers. However, collections from areas far removed from known infestations of fusarium wilt of tobacco were pathogenic for flue-cured tobacco. For example, collections 89, 91, and 95 were made at locations approximately 30 to 90 miles from the nearest known tobacco infestations. These results explain the previously cited field observations by showing that

races pathogenic for flue-cured tobacco do occasionally occur in sweet potato. When, however, they are kept in culture, they generally lose virulence for flue-cured tobacco. The susceptibility of Burley tobacco to all sweet-potato collections was striking (Fig. 1). Complete yellowing of the

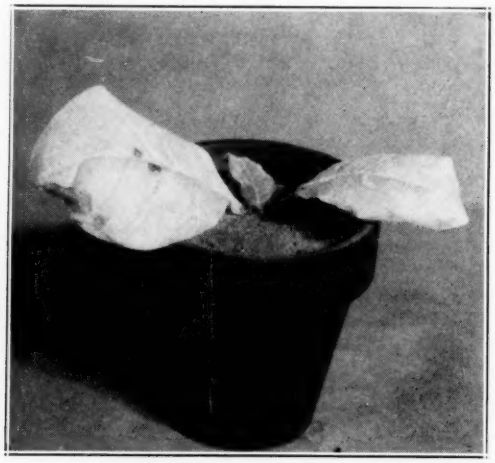

FIG. 1. Susceptibility of Burley tobacco to an isolate from sweet potato. General yellowing of the leaves occurred 5 to 10 days after inoculation and death followed within a few days. Similar results were obtained with all isolates from tobacco, sweet potato, and cotton.

leaves occurred 5 to 10 days after inoculation, and death followed immediately. In several different comparisons, there was a close parallel in the development of symptoms following inoculations on Burley with isolates from sweet potato, nonpathogenic for flue-cured tobacco, and isolates pathogenic for flue-cured tobacco. Burley tobacco, therefore, was as susceptible to races parasitic on sweet potato alone as to races parasitic to sweet potato and flue-cured tobacco.

Results of Inoculation with Fusaria from Cotton

Nineteen collections from cotton were tested, of which 13 came from North Carolina, 5 from South Carolina, and 1 from Georgia. The results of inoculation trials are summarized in table 3. All collections were consistently pathogenic for Burley tobacco and cotton but nonpathogenic for sweet potato. Nearly all collections gave the intermediate reaction on flue-cured tobacco. However, in repeated trials with several isolates which appeared to have the most virulence for this test plant, no typical yellowing of the leaves was obtained. The results were in agreement with observations in the field, previously cited, and show that Burley was susceptible to the cotton *Fusarium*. Flue-cured tobacco was not susceptible to any cotton collection, but from the results with collection 105 (Table 1) it appears that the cotton race may parasitize this type of tobacco to a limited extent. The

TABLE 3.—*Results of inoculations with Fusaria isolated from cotton*

Collection and year originally isolated	Collected at	Results of inoculations on											
		Flue-cured tobacco			Burley tobacco		Sweet potato		Cotton	
		+[a]	±	−	+	−	+	−	+	−
71; 1941	Chadbourn, N. C.	0[b]	2	1	1	0	0	3	3	0
77; 1941	Lumberton, N. C.	0	2	1	1	0	0	3	3	0
78; 1941	Whiteville, N. C.	0	1	1	1	0	0	2	1	1
79; 1941	Fayetteville, N. C.	0	3	0	1	0	0	3	3	0
82; 1941	Chadbourn, N. C.	0	2	1	1	0	0	3	2	1
84; 1941	Kinston, N. C.	0	3	1	2	0	0	4	4	0
87; 1941	do.	0	1	1	1	0	0	2	2	0
88; 1941	do.	0	1	1	1	0	0	1	2	0
96; 1941	Elizabethtown, N. C.	0	1	1	1	0	0	2	2	0
97; 1941	Kinston, N. C.	0	2	1	1	0	0	2	3	0
98; 1941	Elizabethtown, N. C.	0	1	1	1	0	0	2	2	0
100; 1941	Mount Olive, N. C.	0	1	1	1	0	0	2	2	0
101; 1941	Kinston, N. C.	0	0	2	1	0	0	2	1	1
65; 1940	Florence, S. C.	0	0	2	1	0	0	2	2	0
66; 1940	do.	0	13	4	1	0	0	17	16	1
67; 1940	do.	0	1	1	1	0	0	2	2	0
74; 1941	Mullins, S. C.	0	2	1	2	0	0	3	3	0
Armstrong's cultures	South Carolina	0	3	0	1	0	0	3	3	0
	do.	0	3	0	1	0	0	3	3	0
106; 1941	Tifton, Ga.	0	1	1	1	0	0	2	2	0

[a] Explanation of methods used in recording results: + means pathogenic, characterized by extensive discoloration of stele in combination with yellows and wilt on tobacco and sweet potato or wilt only on cotton. ± means intermediate reaction, characterized by severe stunting or slight wilt of green leaves. − means nonpathogenic.

[b] To obtain the total trials on each test plant, add the entries in each cell of the table.

host range of all cotton collections was the same as that of the cotton-virulent race isolated many times from Burley and dark tobaccos but rarely from flue-cured and Maryland types.

EXPERIMENTS ON THE STABILITY OF PATHOGENICITY DURING REPEATED INOCULATION AND REISOLATION FROM RESISTANT PLANTS AND DURING STORAGE

It was noted in many cases that invasion occurred after inoculation, but typical wilt symptoms did not develop. The question arose as to whether the pathogenicity of such weak invaders could be increased by repeated inoculation and reisolation. The question was first investigated with a collection originally isolated from flue-cured tobacco (No. 7, Table 1). This culture was inoculated into and reisolated from cotton 4 times. Attempts to reisolate the culture following the fourth inoculation were unsuccessful, and this series was discontinued with the conclusion that it had not increased in virulence for cotton but was still pathogenic for tobacco and sweet potato. A sweet-potato collection (No. 61, Table 2) gave the intermediate reaction on flue-cured tobacco when originally tested. During 7 passages through this type of tobacco, it varied in virulence from typically pathogenic, with the production of leaf yellowing, to apparently nonpathogenic. The typical symptoms developed following inoculation with the fifth reisolation in July,

1941, during an exceptionally hot period, when maximum temperatures averaged about 100° F. Reisolations gave the usual intermediate or negative reactions in later trials and it was concluded that virulence had not been increased. The culture retained its virulence for sweet potato throughout the 7 passages through tobacco. A cotton collection (No. 66, Table 3) also gave the intermediate reaction on flue-cured tobacco when originally tested. During 7 passages through tobacco, it varied from the intermediate reaction to apparently nonpathogenic but maintained virulence for cotton. From these tests, it was concluded that increases in virulence for resistant plants were not obtained by repeated inoculation and reisolation from them and that pathogenicity was a relatively stable characteristic under the experimental conditions provided. However, there was a partial loss of virulence in many collections during storage. Several isolates from tobacco or sweet potato that were pathogenic on flue-cured tobacco in the first test, were weakly or nonpathogenic in later trials on this test plant, but retained virulence for sweet potato throughout the storage period. An unusually interesting illustration of partial loss in virulence was one of Johnson's cultures isolated in 1937 from dark-fire-cured tobacco in Simpson County, Kentucky. This isolate, when tested in 1941, was nonpathogenic on either type of tobacco, but had retained virulence for cotton.

MISCELLANEOUS INOCULATION TRIALS

Additional pathogenicity trials not reported in earlier sections of the paper were conducted, using the methods previously described. Flue-cured tobacco, sweet potato, and cotton were not susceptible to one isolate from wilted cowpeas collected in a field where fusarium wilt was present on tobacco. Tomato was not susceptible to one collection of the sweet-potato-virulent race from flue-cured tobacco. Tests with Irish potato were not conclusive because of the difficulty of obtaining fusarium-free plants. In two different sets of inoculations, the control plants developed symptoms characteristic of *Fusarium oxysporum* and *F. solani eumartii*, as described by Goss (8). It was clear, however, that Irish potato was not susceptible to all Fusaria from tobacco, because many plants remained healthy after inoculations with several tobacco collections. Cultures of 2 Fusaria from Irish potato were obtained from R. W. Goss of the Nebraska Agricultural Experiment Station. *F. oxysporum* Schl. was nonpathogenic in all trials on flue-cured tobacco, Burley tobacco, cotton, and on root-knot-free sweet-potato sprouts. However, in one test on heavily-galled sweet-potato sprouts, *F. oxysporum* produced symptoms indistinguishable from those caused by sweet potato or tobacco collections. *F. solani* (Mart.) App. and Wr. v. *eumartii* (Carp.) Wr. (Syn. *F. eumartii*) caused no symptoms of wilt on flue-cured tobacco, Burley tobacco, sweet potato, or cotton, but both types of tobacco were stunted and had slightly yellowed leaves. Examination showed stem-rot symptoms similar to those ascribed to *F. tobacivorum* Delac. (6). A second test with the original culture and a reisolation from stem

lesions on Burley tobacco gave similar results. The symptoms differed from "sore shin" of tobacco caused by *Rhizoctonia* sp. in that the lesion was near the base of the stem well below the soil surface and in the area where the larger roots emerged. It is possible that Delacroix worked with a *Fusarium* similar to *F. solani eumartii*.

DISCUSSION

The limitations of host range tests based exclusively on artificial inoculations have been pointed out by the senior writer (15). It was shown that inoculation by artificial methods was not always a reliable method for determining susceptibility to a soil-borne disease. Heavy inoculation with the parasite and its byproducts combined with mechanical injury involved in the operation might break down the mechanism of resistance in plants. In that case the results obtained would not agree with disease occurrence under field conditions. The pathogenicity studies here reported are supported by field observations of an unusual type. The greater part of the observations, those made at Creedmoor and McCullers, North Carolina, and Tifton, Georgia, were made on plots of crop-rotation experiments conducted under controlled conditions to study other diseases. Hence, observations at these locations have the same weight as results obtained in experiments planned specifically for the study of the relation of preceding crop to the occurrence of fusarium wilt of tobacco. The observations of Armstrong (1) were made under less rigidly controlled conditions but are of much value because the tobacco on which wilt occurred was grown in areas where there was no record of the previous culture of this crop. In a study of this type, the writers feel that the accuracy of the inoculation technique is determined by whether the results of pathogenicity trials agree with occurrence of the disease under field conditions. The field observations demonstrated two relationships. First, the occurrence of fusarium wilt of flue-cured tobacco was associated with rotations of sweet potato. Second, the occurrence of *Fusarium* wilt of Burley tobacco was associated with rotations of cotton. Inoculation trials confirmed these two observations by demonstrating that certain isolates of Fusaria from sweet potato caused wilt of flue-cured tobacco and that all isolates from cotton caused wilt of Burley tobacco.

Cross-inoculations with Fusaria from flue-cured tobacco, Burley tobacco, cotton and sweet potato demonstrated that their host range over-lapped (Fig. 2). For example, collections from tobacco were always pathogenic on another plant, either sweet potato or cotton but not on both. Collections from sweet potato were always pathogenic on Burley tobacco, sometimes pathogenic on flue-cured tobacco and never pathogenic on cotton. Collections from cotton were consistently pathogenic on Burley tobacco, mildly pathogenic or negative on flue-cured tobacco, but negative on sweet potato. The wide variability of Fusaria of section Elegans with respect to microscopic and cultural characteristics has been demonstrated by many investigators (12, 14, 17, and others), but pathogenicity within the same "species" was thought to be much more constant. However, the data reported here

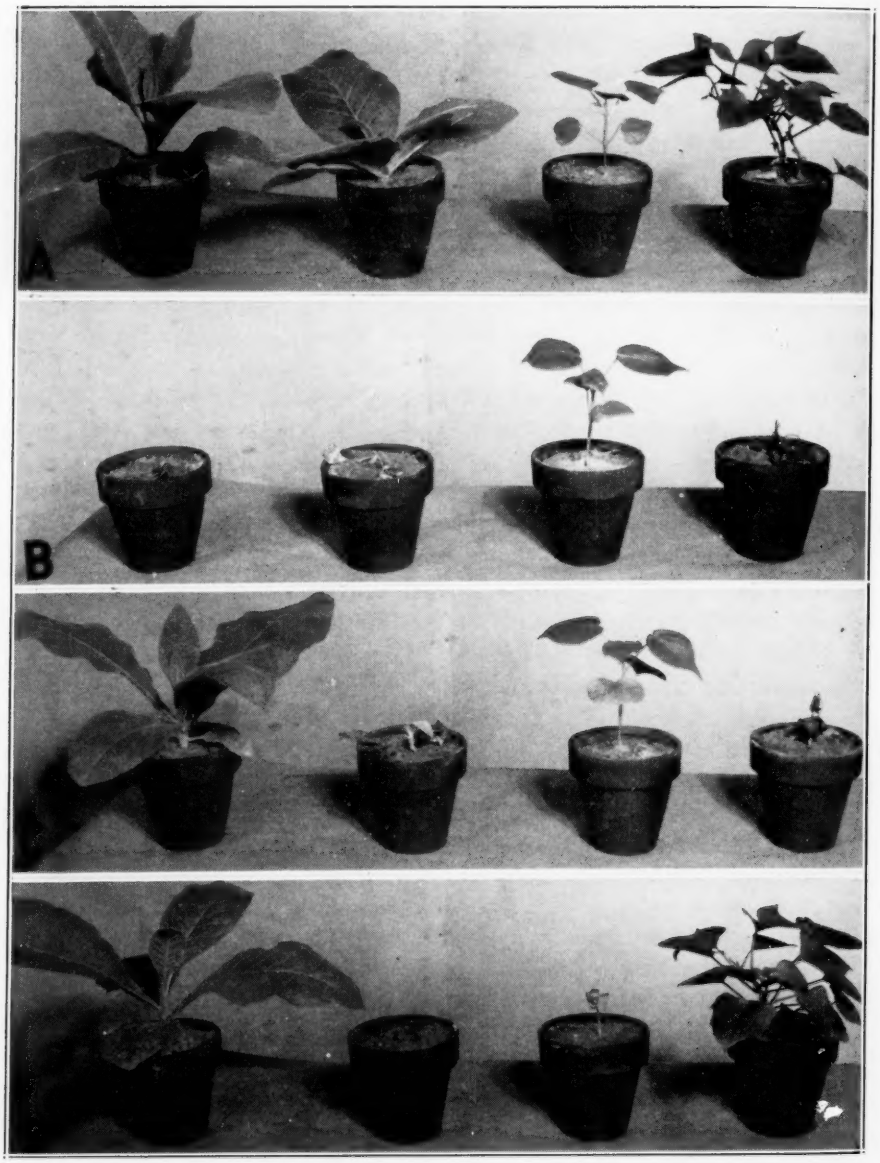

FIG. 2. Pathogenicity of Fusaria from tobacco, sweet potato, and cotton. Left to right: Flue-cured tobacco, Burley tobacco, cotton, and sweet potato. A. Noninoculated controls. B. Isolate from flue-cured tobacco. C. Isolate from sweet potato. D. Isolate from cotton.

and the work of Hansford (9) and Armstrong *et al.* (3)[4] and others show that pathogenicity may not be restricted to the plant species from which a race was isolated.

[4] Since the preparation of this manuscript the investigations of Armstrong and associates have been published in Phytopath. 32: 685–698. 1942.

A reasonable supposition about the origin of the various "species and varieties" of the Elegans section would be that there is one species, considered perhaps as *F. oxysporum* Schl., of more or less universal presence in the soil. It would then follow that various pathogenic races have developed whenever susceptible plants were grown for an adequate period of time. These races may or may not be pathogenically restricted to a single plant species; hence, in order to show the virulence of a single isolate for more than one host, a system based on physiologic races has some merit and requires consideration. For example, 93 of the 94 collections from tobacco, sweet potato and cotton can be separated into 3 physiologic races by the results of cross inoculations on these plants as shown in table 4. The one

TABLE 4.—*Summary of inoculation trials with vascular Fusaria isolated from tobacco, sweet potato, and cotton*

Physiologic race number	Number of isolates obtained from	Results of inoculations on			
		Flue-cured tobacco	Burley tobacco	Sweet potato	Cotton
	Sweet potato				
1	14	−	+	+	−
2	8	+	+	+	−
	Cotton				
3	19	−	+	−	+
	Flue-cured tobacco				
2	20	+	+	+	−
3	1	−	+	−	+
	Maryland tobacco				
2	7	+	+	+	−
3	1	−	+	−	+
	Burley tobacco				
1	1	−	+	+	−
2	11	+	+	+	−
3	6	−	+	−	+
	Dark tobaccos				
2	1	+	+	+	−
3	4	−	+	−	+
3?	1	−	−	−	+

collection not conforming to this system was Johnson's culture, 1937, from dark-fire-cured tobacco in Simpson County, Kentucky. This collection could be considered as race 3 when originally isolated but as having lost virulence for Burley tobacco while kept in storage; therefore, all 94 collections could be said to conform. Many problems, however, such as standardization of inoculation technique, maintenance of virulent cultures, and standardization of genetic resistance of test plants, must be worked out before a system based on physiologic races could be used successfully. Pathogenicity should be the major criterion for the classification of wilt-producing Fusaria, as

pointed out by several workers. Snyder and Hansen (12) proposed a new system based on assumed pathogenicity for single plant species. Wilt of sweet potato was attributed to *Fusarium oxysporum* f. *batatas*, wilt of tobacco to *F. oxysporum* f. *nicotianae* and wilt of cotton to *F. oxysporum* f. *vasinfectum*. Pathogenicity is not that uniform, as shown in table 4.

In considering the practical application of these results, the main conclusions have been verified by field observations and appear to be well founded. It is pointed out that fusarium wilt of tobacco is caused by races of the fungus virulent for sweet potato, cotton, and perhaps other plant species. The use of crop rotations combining sweet potato and all types of tobacco is a hazardous practice because both crops are susceptible to a common race of the parasite. In areas where the disease is present on flue-cured tobacco this combination of crops is exceptionally hazardous because nearly all of the collections from this type of tobacco attacked sweet potato. Fields have been observed in the Whiteville-Chadbourn area of North Carolina in which successive crops of flue-cured tobacco and sweet potato were badly damaged by fusarium wilt. The use of rotations combining cotton and flue-cured tobacco appears to be a safe practice even though most collections from cotton (table 3) were able, to a limited degree, to parasitize this type of tobacco. Burley tobacco was susceptible to all collections from sweet potato and cotton. Therefore, this type of tobacco would probably be subject to fusarium wilt at most locations throughout the Southeast, as observed by Armstrong (1), who found wilt in 6 of 8 fields examined. The situation on Maryland tobacco was not extensively surveyed, but all Maryland collections were virulent for flue-cured tobacco and sweet potato, while one collection made in the cotton belt at Florence, South Carolina, was virulent for cotton. It appears, then, that fusarium wilt of all types of tobacco may be caused by a race of the fungus pathogenic on sweet potato, but on Burley and dark-fired tobaccos it is also caused, in many instances, by a different race of the fungus, which also is pathogenic on cotton.

SUMMARY

Field observations suggested a close relation between Fusaria causing wilt of tobacco, sweet potato, and cotton.

Pathogenicity trials with 53 collections from tobacco showed that fusarium wilt of tobacco in these cases was caused by races of the fungus virulent on sweet potato or cotton but not on both. The race pathogenic on sweet potato was obtained in nearly all collections in North Carolina, South Carolina, and Georgia from flue-cured tobacco and in Maryland from Maryland tobacco. Races pathogenic on sweet potato or cotton were collected in approximately equal numbers in Kentucky from Burley and dark tobaccos.

Pathogenicity trials with 22 collections of *Fusarium* from sweet potato showed that all were pathogenic on Burley, some but not all pathogenic on flue-cured tobacco, and all negative on cotton.

Pathogenicity trials with 19 collections of *Fusarium* from cotton were all pathogenic on Burley tobacco, slightly pathogenic or negative on flue-cured tobacco, and negative on sweet potato.

All 94 collections could be grouped into 3 physiologic races on the basis of results of inoculation on flue-cured tobacco, Burley tobacco, sweet potato, and cotton. The data show that more emphasis should be given to pathogenicity in the classification of Fusaria of the Elegans section.

In conclusion, it is pointed out, that the use of crop rotations combining the growth of sweet potato and all types of tobacco is a hazardous practice. Both crops are susceptible to a wide-spread race of *Fusarium*.

The growth of Burley and dark tobaccos in rotation with cotton also appears to be a hazardous practice. However, flue-cured tobacco may be safely grown in rotations with cotton.

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A BROWNING REACTION TO STEM RUST IN WHEAT[1]

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(Accepted for publication September 7, 1942)

Reaction to rust infection has always been considered a relatively stable character in a cereal variety, and yet there may be considerable variability in abundance of sporulation, degree of chlorosis of host tissues, extent of mycelial development within tissues, and in the pigmentation surrounding the rust pustule or point of infection. In some instances the variability in reaction of certain wheat varieties to *Puccinia graminis tritici* Eriks. and Henn. is so extreme as to make difficult a placement of reaction type in one of the five classes described by Stakman and Levine (15). Sporulation may vary greatly (Fig. 1, A and C), and pigmentation around the point of infection (Fig. 1, A, B, and C) may be so dark as to distract attention from the usual slight chlorosis of host tissues (Fig. 1, D). Such a lack of sporulation and a deep pigmentation seem to indicate greater resistance to rust; and the present paper describes the frequency of this particular type of reaction to stem rust, the factors responsible for it, and its histological aspects.

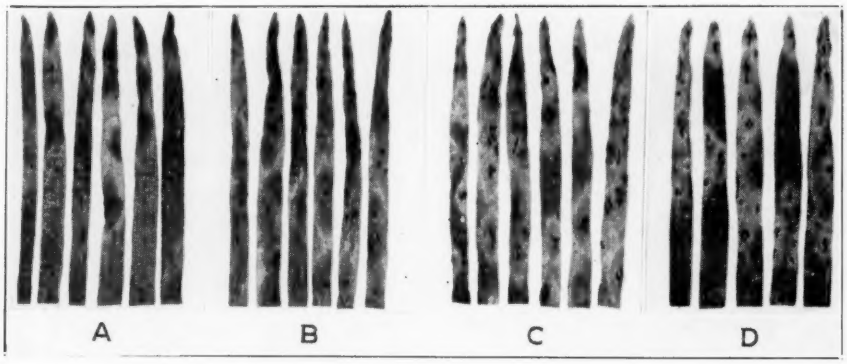

FIG. 1. Variability in reaction of Thatcher wheat to race 34 of *Puccinia graminis tritici*. A. Severe browning about point of infection and a trace of sporulation. B. Moderate browning and fair sporulation. C. Moderate browning and good sporulation. D. Normal reaction without browning, but with slight chlorosis about pustules.

FREQUENCY OF BROWNING IN CERTAIN WHEAT VARIETIES

This reaction, which conveniently may be called a browning reaction, because the deep brown appearance of tissues about the point of infection is its outstanding characteristic, occurs rather often in seedlings of certain

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wheat varieties, and seems never to occur in others. Vernal emmer, Arnautka, Kubanka, Kota, and Kanred are subject to the browning reaction; and selections from them or their hybrids often have the same reaction. This is true for Acme, a selection from Kubanka, and for the following hard red spring wheat varieties derived by hybridization from parental material subject to browning: Ceres, Rival, Reliance, Thatcher, Hope, and Pilot. The browning reaction may be frequent in the 14-chromosome *Triticum timopheevi* Zhuk, and in einkorn, as well as in the 14- and 21-chromosome species of *Triticum*. On the other hand, the reaction never has been observed in Marquis or in Little Club. Nevertheless, the browning reaction cannot be considered a varietal character, because its expression depends on the race of rust involved and the external environmental conditions incident to rust infection.

ASSOCIATION OF THE BROWNING REACTION WITH CERTAIN RUST RACES

During the 3 or 4 years that the browning reaction was observed closely, certain physiologic races of *Puccinia graminis tritici* were associated with its occurrence more often than others (Table 1). Whenever collections of

TABLE 1.—*Host–parasite combinations in which the browning reaction frequently occurs in different varieties of wheat*

Race of *Puccinia graminis tritici*	Susceptible host variety	Resistant host variety
11	.....	Vernal
15A	Acme, Arnautka, einkorn, Hope, Kota, Kubanka, Thatcher, Vernal	Khapli
15B	Acme, Arnautka, einkorn, Kota, Kubanka, Thatcher	.....
21	Ceres	.....
34	Acme, Arnautka, Ceres, Hope, Kanred, Kota, Kubanka, Thatcher, Rival, Pilot	einkorn, Khapli, Vernal, *Triticum timopheevi*
38	Acme, Arnautka, Kubanka	Khapli
40	.....	einkorn
42	Khapli	.....
56	.....	einkorn, Arnautka, Vernal
79	.....	Arnautka
147	Kubanka	.....
166?	.....	Arnautka, Mindum, Spelmar

race 34 were cultured in the greenhouse, the browning was frequent and present in a large proportion of the differential *Triticum* varieties inoculated: at least 10 different collections of race 34, from various parts of the United States and from Scotland apparently induced the browning reaction in a selected lot of varieties under favorable environmental conditions. Race 34, whether collected from wheat or from barberry, seemed capable of inducing this particular variation of a normal stem rust reaction in more different wheat hosts and in more experimental instances than any of the other rust races tested.

The browning reaction was rather frequent in certain hosts rusted by a race 15 collected from Japan (a biotype designated 15A by Loegering and Stakman (10)) in 1942. Fewer hosts responded thus to the Japanese collection than to race 34, but incidence of browning over a period of many months approximated that with race 34. In later experiments another collection of race 15, corresponding to the biotype 15B of Loegering and Stakman (10) induced browning as effectively as did the Japanese collection.

When other races of stem rust were involved, fewer varieties had the browning reaction and its frequency in different experiments was lower. On several occasions infected tissues of Vernal emmer were deeply pigmented when rusted by a collection of race 11 from Chile. Kubanka seedlings sometimes reacted thus when rusted by races 38 or 147, and einkorn responded likewise to race 40. Other isolated instances of this variation in reaction occurred in response to races 21, 42, and 56; but it was never observed in any host rusted by races 17, 36, 59, or 69. In all circumstances, too, it was evident that environment played as great a role as did the cereal hosts and rust races concerned in the reaction.

TEMPERATURE AS THE PRIMARY ENVIRONMENTAL FACTOR RESPONSIBLE FOR BROWNING

High temperature, above 28.5° C., was the one factor of the environment directly responsible for browning in certain wheat varieties inoculated with rust. Furthermore, the degree of pigmentation varied directly with the length of time the temperature remained above 28.5° C. Brief exposure of inoculated and infected seedlings to high temperature was sufficient to initiate the reaction, so that a slight browning was evident to the eye within a few days of the exposure. A deeper host coloring and reduced sporulation of the rust fungus followed longer and repeated exposures. If temperatures were high during the incubation period, within 2 to 4 days after inoculation, and before pustules were formed, the pigmentation was severe and fairly extensive, and there was less sporulation or it was almost inhibited (Fig. 1, A). Later exposure to high temperature, after pustule formation, seldom brought about a severe reaction, unless the exposure was prolonged.

Moderately high atmospheric humidity accelerates the expression of browning reaction, but it cannot induce the reaction without the primary stimulus of high temperature. Other environmental factors—light, nutrient relations, soil moisture—produced no noticeable effects. They were relatively unimportant when compared with temperature and with atmospheric humidity.

Since the browning seemed to indicate greater resistance to stem rust at high temperatures, the infected tissues were examined for indications of internal tissue changes or changes in development of the parasite.

HISTOLOGY OF THE REACTION

Numerous materials were prepared and sectioned in several different ways. The most satisfactory was the simplest: sectioning fresh material on the freezing microtome and mounting sections in lactophenol without staining. The pigments were retained better with such treatment than when material was fixed and killed, dehydrated with alcohols, and embedded in paraffin, then stained subsequent to sectioning.

A great abundance of mycelium was formed in most of the lesions, so that hyphae permeated the host tissues for some distance beyond the rust pustule. In the browned area of most hosts (Fig. 2) most of the rust haustoria had become dark and thick-walled and were so numerous as to account for a large part of the browning evident in a surface view of the

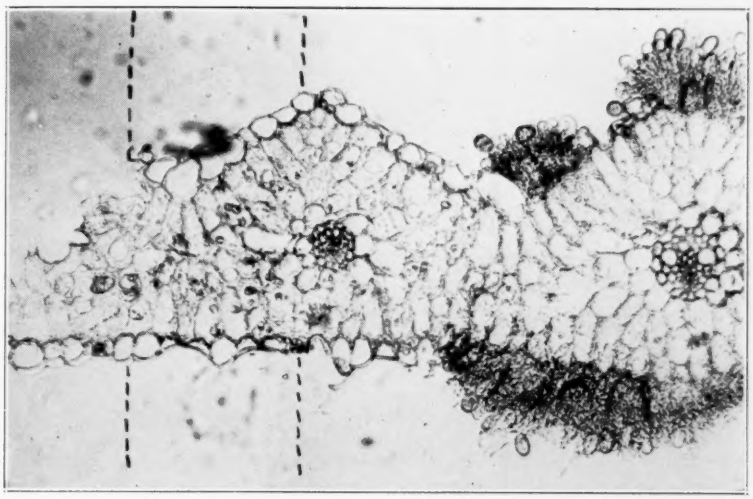

FIG. 2. Cross section of an Arnautka seedling with a moderate browning reaction to race 34 of *Puccinia graminis tritici*, showing an abundance of mycelium throughout the leaf tissue and a number of transformed haustoria (in the area between the dotted lines) at some distance from the rust pustule but coinciding with the browned area seen in a surface view of the leaf.

leaf. While the transformed haustoria were the most conspicuous features of the browned area they were not solely responsible for browning. Near the outer margins for mycelial development the cell walls were discolored in groups of host cells, usually a group of 5 to 15 cells not far removed from a vascular strand in the leaf. Host cell walls were brown and slightly thickened in many cases. If the sections were run through the alcohols the discolored walls were far less evident, and it was only from the appearance of freshly cut sections in lactophenol that we could judge how important the wall discolorations were in enhancing the "browning" effect seen in surface view of the leaf. One other thing contributed to the effect, the fact that the tips of some of the intercellular rust hyphae were discolored and sometimes transformed and ensheathed as were the haustoria. In a

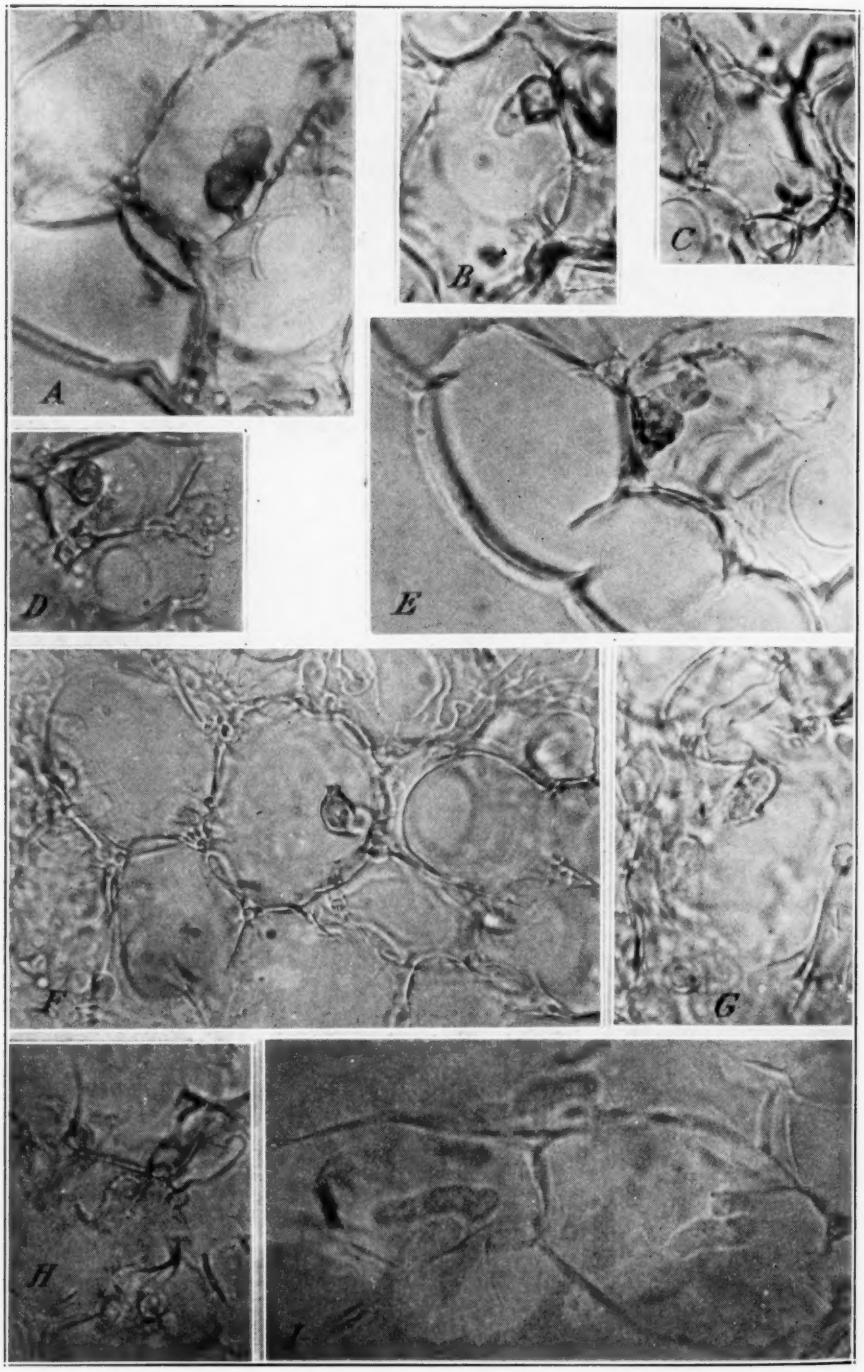

FIG. 3. Photomicrographs showing histological details associated with the browning reaction of wheats to *Puccinia graminis tritici*. *A*. Two haustoria of race 34 encysted within a host cell of Kubanka wheat, one completely encased in a thickened and darkened

few cases these hyphae were primarily responsible for the browning and were conspicuous in tissues immediately adjacent to the vascular bundles.

Studying the transformed haustoria more closely we saw that there had been an attempt at encystment, with results similar in many respects to what has been described by other authors (1 through 6, 12 through 14, and 16) for rust infections in a resistant host tissue or in an aging rust infection. In a cross section of a wheat seedling susceptible to stem rust the haustoria usually are delicate, elongate, sac-like protrusions into the host cell and host protoplast, connected to the intercellular rust hyphae by slender strands of protoplasm (Fig. 3, *I*). These normal haustoria also were present in seedlings with a browning reaction, but few in number in comparison with those that were partly to completely ensheathed. The formation of the thick sheath began at the base of the haustorium and about its neck, the slender protoplasmic thread joining the body of the haustorium to its mother cell (Fig. 3, *C* and Fig. 4, *D*). The sheath gradually enclosed most or all of the haustorium (Fig. 3, *B* and Fig. 4, *E* and *G*), but never was observed about the haustorium mother cell.

Pigmentation of the sheath varied considerably: it was sometimes dark brown to black in the collar-like sheaths that were formed early (Fig. 3, *C*) or in a completely encased haustorium (Fig. 3, *A*); it might be dark brown near the base of the haustorium body and less intense in the other parts (Fig. 4, *D*, *F*, and *G*); or it might be lacking in a part or all of the enclosing sheath (Fig. 4, *E* and Fig. 3, *G* and *H*). In a few sections there were occasional haustoria of exactly the same golden brown as the normal urediospores in nearby pustules in the same sections (See Fig. 4, *I*).

Ensheathed haustoria varied greatly in shape, very few of them being so elongate as normal haustoria unless the sheath itself was limited or slow in its development (Fig. 4, *D* and *E*). Some were nearly spherical (Fig. 3, *A* and *D;* Fig. 4, *F*), especially in cases of hosts resistant to the rust race present, or in cases of rapid and severe browning. By far the greatest number were ovoid, with a definite constriction at the middle (Fig. 3, *B*,

membrane, the other only partially encased. Haustorial mother cells were not affected. *B*. A single haustorium of race 15A partially encysted within a host cell of Acme wheat. Thickening has not yet occurred about the tip of the haustorium. *C*. An early stage in the sheathing of an haustorium of race 15A in Acme wheat. The very thick and dark saucer-shape sheath started at the base of the haustorium and around the slender strand of protoplasm between haustorium and its mother cell, but the tip of the haustorium was not changed. *D*. An encysted haustorium that was nearly spherical, a form encountered often when a resistant host (einkorn) had a browning reaction to race 34. *E*. An abortive teliospore formed by a hyphal tip of race 34 just beneath the epidermis of Kubanka wheat. Two cells were evident, the tip cell having the thick, dark, cap-like structure of a normal teliospore. *F*. Teliospore-like haustorium of race 15A associated with browning of Kubanka wheat. The tip of the haustorium had been encased in a thick, dark, papillate sheath similar to the wall of a teliospore, and the middle of the haustorium seemed to be constricted. The base of the haustorium, corresponding to the basal cell of a teliospore, was not in focus in the photograph. *G* and *H*. Urediospore-like haustoria of race 15A in cells of Acme wheat. The thick sheaths about the haustoria were not deeply pigmented but were rough and resembled the echinulate walls of normal urediospores. The spore mother cell and the hypha shown in B were not affected and had smooth walls. *I*. Normal thin-walled haustoria of race 34 in host cells of Kubanka seedlings that did not have a browning reaction.

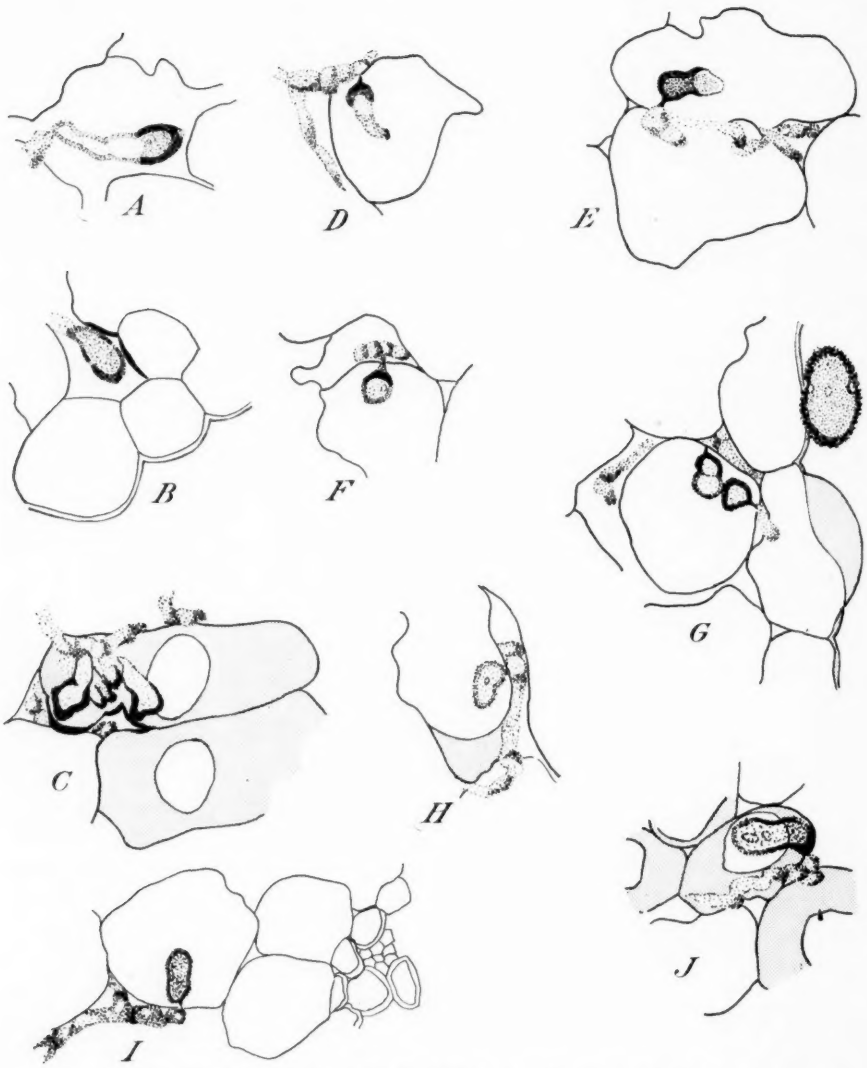

FIG. 4. Camera-lucida drawings of histological details associated with the browning reaction of wheat to *Puccinia graminis tritici*. *A*. Tip of a hypha of rust race 15A in an intercellular space in a Kubanka seedling. Constrictions in the hypha and its thick sheath with dark pigment and papilla at the tip gave it the appearance of an abortive teliospore. *B*. Hyphal tip of race 15A with thick, dark sheath in an intercellular space just beneath the epidermis of an Acme seedling. Host cell walls adjacent to the hyphal tip were somewhat thickened and dark. *C*. A mass of hyphae of race 34 lying on a host cell of Arnautka wheat. Two of the hyphal tips were thick and dark, slightly constricted, and somewhat papillate, so that they resembled abortive teliospores. Host cell walls near the transformed hyphal tips were thickened and dark. *D*. An early stage in the tranformation of an haustorium of race 15A in Kubanka wheat. Thickening and darkening of the sheath began at the base of the haustorium, while its tip was not affected until later. *E*. A later stage in the transformation of an haustorium of race 34 in Kubanka wheat. A thick sheath encased the entire haustorium, but pigmentation was evident over only $\frac{3}{4}$ of the sheath. *F*. A spherical, encysted haustorium of race 15A in a cell of Kubanka wheat, a type frequent in cases of severe browning. *G*. Two encysted haustoria of race 34 within a host cell of Kubanka. One haustorium resembled an abortive teliospore and appeared to

F, G, H, and Fig. 4, *E, G, H, I, J*), so that they called to mind abortive urediospores or teliospores on a miniature scale.

The sheath was smooth about most of the haustoria; but if pigment was lacking, or present in only a portion of the thick sheath, there appeared to be echinulations or a verrucose roughening of the sheath surface (Fig. 3, *G, H,* and Fig. 4, *H, J*). On rare occasions in a sheath almost devoid of pigment there were thinner areas in the sheath resembling in placement and proportionate area the germ pores of normal urediospores. Unfortunately, such cases were rare, and it was not possible to secure a photograph of such an haustorium.

The many similarities between ensheathed haustoria and rust urediospores or teliospores—golden to dark brown in lactophenol, shape and constriction about the middle, character of wall markings, and indications of thin areas—lead us to think that the haustorial sheath is a fungus rather than a host deposition. How long an ensheathed haustorium remains alive and how sheathing affects the permeability of the haustorial membrane and the subsequent nutrition of the rust parasite remain to be explained, and some research along these lines has been undertaken by F. S. Thatcher. We have been unable to separate an ensheathed haustorium from its host cell, and, so far as we can tell, there is no evidence that the haustorium, once it is ensheathed, ever resumes growth when favorable conditions return. We do not know that it actually is an abortive spore.

The tips of many of the intercellular rust hyphae had undergone changes similar to those in the haustoria within the cells. The hyphal membranes were greatly thickened, particularly at the very apex of a hypha, so that there was a papillate tip resembling that of a teliospore of stem rust (Fig. 3, *E;* Fig. 4, *A* and *C*). Pigment often was unevenly distributed in the thickened membrane (Fig. 3, *E;* Fig. 4, *A, B,* and *C*), but the greatest amounts were usually near the apical end. The swollen hyphal tip generally was constricted once or twice at short distances from its apex (Fig. 4, *A* and *C*). Sometimes a septum divided the swollen tip (Fig. 3, *E*). Again, the resemblance to teliospores was striking, except in the matter of size and in completeness of development. Host cell walls adjacent to such transformed hyphal tips sometimes were slightly thickened and discolored (Fig. 4, *B* and *C*), but this was not always true. The intercellular spaces immediately beneath the epidermis or contiguous to a vascular bundle were more often occupied by these changed hyphal tips than were the spaces in other parts of the leaf mesophyll, but there was never such an aggregation of hyphae as one might expect for the formation of a normal uredium or telium.

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be divided by a cross membrane. Size of haustorium may be compared with the normal urediospore lying outside the epidermis of the host. *H.* Haustorium of race 15A with thick, rough wall, resembling an abortive urediospore more than a teliospore. Pigmentation was not especially noticeable. *I.* Urediospore-like haustorium of race 34 on Pilot wheat. In lacto-phenol this haustorium was golden brown as were the normal urediospores. *J.* Extremely large haustorium of race 34 in Kubanka wheat, with thick, dark sheath.

OCCURRENCE OF BROWNING IN OLDER PLANTS

The browning reaction has been observed in older plants of many of the varieties included in the experiments with seedlings. Such observations, however, were usually incidental to other experimental arrangements and results. In the few sections cut from older materials the histological features corresponded with those found in seedlings of the same varieties.

The senior writer (8) first called attention to deep brown or black pigmentation and restricted sporulation in plants of Hope wheat inoculated with race 21 of *Puccinia graminis tritici* and kept at high light intensities or at high temperatures. Unfortunately, less attention was paid at that time to the histological study of early stages of infection, and in practically all of the material prepared from late stages of infection there were many dead host cells and dense mycelial infections that failed to sporulate or that produced pustules closely confined within the host tissues and with abortive and distorted spores. The rust collection used at that time is no longer available and it has been impossible to check and extend the previous work. It is believed, however, that the phenomenon encountered at high light intensity and high temperature is similar to but more severe than that herein described.

Similar pigmentation and discoloration were described by McFadden (11) for Hope and related wheats inoculated with races 11, 17, and 21 of *Puccinia graminis tritici* and grown in field plots in Texas in 1936 and 1937. McFadden uses the term "brown necrosis," because he found many of the plant cells were killed whenever the discolored blotches appeared. As early as 1915 he observed the reaction in Vernal and Yaroslav emmers, and later on Acme durum also. He reported no histological studies and no work with seedlings.

Johnson and Hagborg (9) also studied discolorations in wheat and found that some were caused by stem rust. Races 34 and 120 of *Puccinia graminis tritici*, particularly the latter, caused brown necrosis on heads and peduncles of Apex wheat. The discolorations were observed in varieties derived from crosses of Marquis and Reward with Hope, H 44, and Pentad. In glumes of Renown wheat examined histologically the stem rust had formed appressoria and entered the stomata but very little mycelium had developed beyond the substomatal cavity and the parenchyma cells appeared to be highly hypersensitive to rust. These authors also reported no work with seedlings.

It is noteworthy, however, that these browning reactions in relation to stem rust infection are most conspicuous by their presence in the hot and dry decade 1930–1940. Observations of Hart and Zaleski (8) covered the years 1932 and 1933; the extensive observations of McFadden (11) were made in 1936 and 1937; and Johnson and Hagborg (9) reported discolorations generally prevalent at Winnipeg on glumes and lemmas of many newly developed wheat varieties in 1935 and 1938; and the research herein reported was begun in 1936 and completed in 1940. From conversations

with E. C. Stakman, it also is apparent that this type of reaction was frequent during the identification of physiologic races of wheat stem rust collected during the physiologic-race survey of 1934, a year marked by exceptionally high temperatures in Minnesota. The durum wheats in the different series, especially Acme and Arnautka, often were less susceptible than expected. Race 34 of *Puccinia graminis tritici* generally was reisolated from poorly sporulating and off-color pustules on Acme and from the seemingly resistant reaction types on Arnautka. In 1934, when race 34 constituted 22 per cent of the stem rust races identified from collections on wheat, and often during hot periods of later years, the browning action might easily have been used as a supplementary means of identification when race 34 was mixed with certain other races in a collection. Whether browning will be of general occurrence on a number of hosts in the future remains to be seen.

GERMINATION OF UREDIOSPORES AT HIGH TEMPERATURE AND IN HOST PLANT EXTRACTS

Since race 34 of *Puccinia graminis tritici* was more often associated with browning than any of the other races, we tested the effect of high temperature on the germination of its urediospores to note any tendency toward encystment of the fungus outside the host plant when urediospores germinated in distilled water or in extracts from certain hosts.

Plant sap was expressed from healthy, 7-day-old, Kubanka seedlings that had been frozen quickly at – 3° C. The sap was passed through a sintered glass filter and diluted with sterile distilled water to the desired concentration (1:100, 1:1000, 1:10,000, 1:100,000, and 1:1,000,000). Checks were in hanging drops of sterile distilled water.

At 27° C. the germ tubes were long and sinuous and often branched, both in distilled water and in the filtrates. At 33° C., however, a large proportion of the germ tubes grew to a length of 2 or 3 times the urediospore length but then rounded up to form a bulbous portion (Fig. 5, *B* and *C*). After a short time the germ tube resumed growth from a pore-like opening in the bulbous encysted cell. Such abnormal germ tubes were fairly abundant in all dilutions of the filtrates and in distilled water at 33° C. Frequently the tips of longer germ tubes were transformed to spore-like bodies at the high temperature. In figure 5, *A*, a germ tube, 5 to 6 times the urediospore length, had a tip with a very thick wall, dark-brown pigment in the thickened wall, a papillate thickening at the apex, and what appeared to be two separate cells. Resemblance to an abortive teliospore is very pronounced. Ezekiel (7) found structures of the same sort produced by germinating urediospores of several different races of *Puccinia graminis tritici* and also other rusts.

DISCUSSION

The browning reaction well illustrates the possibility of rather wide variations in reaction to stem rust. While in resistant hosts the browning

reaction is only an added indication of resistance, in a susceptible host a severe browning reaction may signify a definite shift from susceptibility to resistance.

The results of such variations may be more far-reaching than would seem evident from limited experimental work. It is possible that such a variation as this browning reaction has been one of the factors responsible for the fact that race 34 no longer plays an important part in the stem rust epidemics in the Mississippi Valley. In 9 of the 12 years from 1930 to 1941, inclusive, race 34 constituted less than 5 per cent of the stem rust population,[2] although many of the varieties of winter wheats, hard red

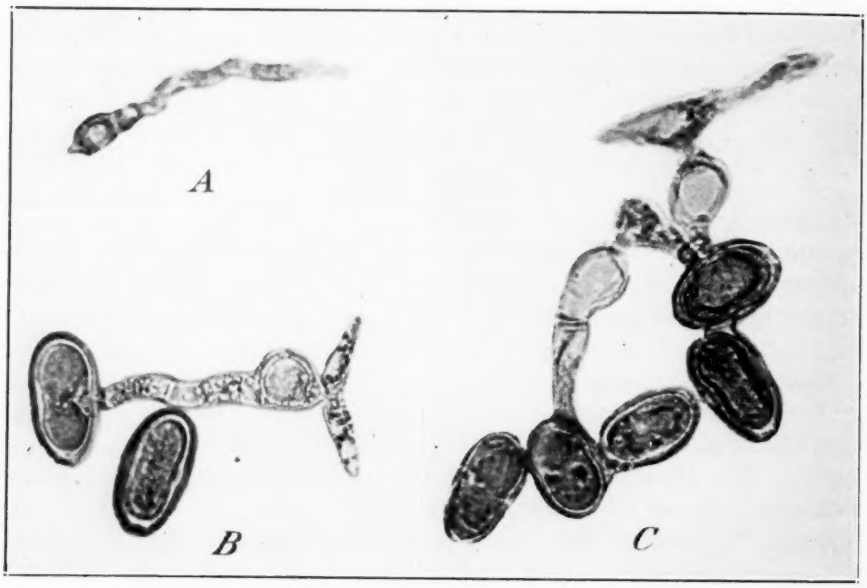

FIG. 5. Photomicrographs of the germination after 18 hours of race 34 of *Puccinia graminis tritici* at 33° C. in filtrates of 7-day-old Kubanka seedlings. *A*. Tip of a germ tube that produced an abortive teliospore, in concentrated filtrate. *B*. Germ tube from a urediospore, with swelling and an attempt at encystment in the third cell formed, and with renewed growth of a slender, branched, thin-walled germ tube from the swollen cell. In a 1: 1,000,000 filtrate. *C*. Two germ tubes in which the second cell formed had swollen and from the swollen cells new germ tubes had grown out. The swollen cells seemed to be empty of protoplasm. In a 1: 10,000 filtrate.

spring wheats, and also durums are susceptible to this race. In the spring wheat region race 34 survived the hot, dry years 1930 through 1934 as well as or better than many of the other stem rust races, but by 1934 race 56, which Cassell[3] found more tolerant of high temperatures than most of the other rust races, predominated. Since the entire decade 1930–1940 may

[2] We are indebted to E. C. Stakman and W. Q. Loegering, Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, for data from their unpublished reports.

[3] Cassell, R. C. Factors affecting the distribution of physiologic races of *Puccinia graminis tritici*, Eriks. and Henn. University of Minnesota. Ph.D. thesis, June 1938. (Unpublished.)

be characterized as hot and dry for the Mississippi Valley in general, it is not surprising that the heat-tolerant race 56 supplanted the other races to so great an extent. Race 34 was not immediately eliminated with the rapid rise of race 56, however, and in 1934 and again in 1935 there were surprisingly large proportions of race 34—22 per cent and 18 per cent, respectively. The explanations for the persistence of race 34 lie in the environment during those years and in the earliness with which rust developed. In 1934 stem rust appeared 7 to 10 days earlier than usual; and early in a growing season when temperatures usually are low and moisture is sufficient race 34 undoubtedly has as good a chance for survival as other races. With 7 to 10 days start in 1934 race 34 might have multiplied enough to become an important factor in the stem rust epidemic. Watson (17) found that race 34 was a good competitor when grown in association with other races on Little Club or on a Soft Federation wheat from Australia. It is true that the incubation period for race 34 on certain susceptible hosts seems from $\frac{1}{2}$ to 2 days shorter than for other races. Such an advantage undoubtedly contributed to the build-up of early season inoculum of race 34. The spring of the following year, in which race 34 was important, was cold and wet; cloudly weather prevailed until the end of June. Even after hot weather set in towards the end of June, there were frequent showers to modify the high temperatures and to contribute to stem-rust development in plants that may have been inoculated during the cooler, cloudy days of mid-June.

The fact that many of the new rust-resistant hybrids and much of the breeding material seem subject to browning may help in further reduction of certain races in the future rust populations if we have more growing seasons marked by extreme heat. If, however, we have several seasons of moderate temperature we might experience an increase of certain races involved in browning reactions, provided susceptible hosts are at hand for their increase.

Study of the browning reaction also brings up many questions as to adjustments of the obligately parasitic rust fungi to their gramineous hosts, and some of these were previously raised in Rice's (12, 13) excellent articles. It is hard to say whether the haustorium is actually the specialized structure we have heretofore considered so characteristic of obligate parasites or whether it is somewhat more like the normal intercellular rust hyphae. Both seem capable of the same reactions under the adverse conditions described in the present work. The indications here are that the sheathing of the haustoria or the hyphae occurs by the same process that brings about normal sporulation of the rust fungus. It is still not known whether changes in permeability, shifts in oxidation relationships, increases in the activity of certain other enzymic systems, or other specific metabolic changes may provoke sporulation or encystment of the fungus. And in cases of normal sporulation, as well as of protective sheathing, we cannot tell what determines whether urediospores and urediospore-like bodies will be produced or teliospores and teliospore-like bodies will appear.

SUMMARY

The browning reaction consists of a deep brown discoloration of wheat host tissues about a stem rust infection center, usually accompanied by reduced sporulation of the rust.

Browning has been associated with only certain wheat varieties infected with certain races of stem rust and under certain environments.

Temperatures above 28.5° C. constitute the primary environmental factor responsible for browning. High relative humidity enhances the effects of high temperature.

Rust hyphae and rust haustoria become ensheathed and transformed. Resemblance of the transformed parts to urediospores and teliospores is striking in several respects: pigmentation, shape and constriction about the middle, character of wall markings, indications of a band of thin areas in the wall, and papillate apical thickenings.

Host cell walls in a browned area may be discolored and slightly thickened.

There is some tendency toward spore-like encystment of germ tubes when urediospores of race 34 of *Puccinia graminis tritici* germinate at high temperature.

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PHYSICAL CHARACTERISTICS OF BORDEAUX MIXTURE IN RELATION TO ITS QUALITIES

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The common belief among plant pathologists that the physical characteristics of the precipitate of Bordeaux mixture influence the fungicidal value of the spray is typified in the following statement by Lutman (8). "The peculiar physical structure of the copper compound which is precipitated is the characteristic which gives to Bordeaux mixture a great share of its value. . . ."

According to a number of investigators (1, 2, 5, 7, 11) the concentration of the constituents at the time of mixing influences the suspension qualities of the precipitate. If, in making 8–8–100 Bordeaux, for example, the copper sulphate and lime in stock preparations, which commonly contain from 0.5 to 1 lb. of constituent to each gallon of water, are first mixed and then diluted with the requisite amount of water, the resulting precipitate settles more rapidly than that formed when the constituents are each diluted with one-half the required amount of water and then mixed. A difference in rate of settling, therefore, indicates the physical characteristics, in this case the specific gravity, of the precipitate.

From studying formation of precipitates under different conditions, Swingle (10) concluded that the combination of relatively concentrated preparations produced more of the "closed cells"—spherical sacs of lime suspension surrounded by membranes of the blue precipitate—than the combination of more dilute preparations. Lutman (8) came to similar conclusions, particularly with reference to the effect of a concentrated copper sulphate solution. He reported that, when undisturbed, the membranes surrounding closed cells thickened until considerable force was required to break them, and that rate of thickening was greatest when a relatively concentrated copper sulphate solution was used.

Swingle (10) believed that the precipitate of a properly prepared Bordeaux mixture contained much imbibed water, a condition that would contribute to its bulkiness. In certain aspects this has been confirmed by Reckendorfer (9), who found that the precipitate swelled measurably in water. The ratio of lime to copper sulphate, furthermore, affected the swelling capacity, inasmuch as increasing the lime to copper sulphate above 1:1 was accompanied by a decrease in swelling.

Lutman (8) believed that agitation broke the precipitation membranes which surround closed cells, and aided in the reaction between the copper sulphate and lime, and thus affected both the quantity and thickness of precipitation membranes. Hawkins (5) showed a definite increase in suspension qualities when Bordeaux prepared from a dilute copper sulphate solution and a concentrated lime suspension was shaken vigorously. In such

instances one of the primary functions of agitation is preventing the local concentration of one constituent by quickly dispersing it throughout the larger volume of liquid containing the other.

Although settling rate and to some extent volume of the precipitate have been used to indicate quality of Bordeaux (1, 5, 6, 7, 11), search of the literature shows but few instances where attempts were made to determine the relation between the physical characteristics of the precipitate and either its general fungicidal performance or the factors that influence this performance, *i.e.*, the toxicity of the precipitate to fungus spores, amount and distribution of deposit over the sprayed surface, or resistance (tenacity) of the deposit to removal by rain. Without giving his evidence Cunningham (3) asserted that only by mixing the two constituents in a concentrated form did he obtain "unsatisfactory results." Under greenhouse conditions Yarwood (12) found but little difference between the immediate protective and eradicant values of Bordeaux mixtures prepared from concentrated and diluted stock preparations of copper sulphate and lime, when applied in control of powdery and downy mildews of a number of plants. In the case of bean rust, however, the mixture prepared from diluted preparations was somewhat superior to that prepared from concentrated preparations.

The primary purpose of this article is to report studies on the weather resistance of Bordeaux mixtures that varied in physical characteristics because of differences in method of combining copper sulphate and lime. Information of this type is particularly pertinent to effective prevention of a peach and apricot disease caused by the fungus *Coryneum beijerinckii*. The fungus attacks twigs and dormant buds in winter. To prevent this infection an application of Bordeaux, 10–10–100, is given in the autumn subsequent to leaf fall. As this application is expected to protect the buds and twigs throughout the winter (14 to 16 weeks) when heavy rains occur, the tenacity with which the deposit resists removal determines to no small degree the control obtained.

Other factors, such as the fineness and the calcium carbonate content of the lime, are known to influence the suspension qualities of Bordeaux mixture (4, 6), but the relation of these to tenacity of the deposit was not studied in the present work.

SETTLING, DEPOSITION, AND TENACITY OF BORDEAUX MIXTURE

The writer's studies on the tenacity of Bordeaux mixtures prepared from diluted and concentrated constituents were conducted in the field in December, 1941, and January, 1942. The trees used for the purpose were 4-to-5-year-old peaches and apricots of uniform size. The surfaces on which the tenacity of the spray was determined were those of twigs produced in 1941 only.

After the spray was applied, at a pressure of 300 pounds, and had thoroughly dried, 3 samples of twigs, 4–6 inches long, were taken from each

tree. These were weighed immediately and placed in glass jars with tight-fitting glass tops. Two hundred cc. of nitric acid solution (25 cc. of C.P. nitric acid, sp. gr. 1.416, per liter) was run into each jar, the tops fixed in place, and the jars rolled for 10 minutes on a mechanical rolling device. The solution was then filtered and aliquots were taken for copper determination by the iodometric method.[1]

In test 1, the Bordeaux made with diluted constituents, and hereafter known as mixture 1, was prepared by pouring a suspension containing 1.6 lb. of slaked quicklime into the spray tank containing 15 gal. of water. With the agitator running 1 gal. of a solution containing 1.6 lb. of copper sulphate was then poured in and the required amount of water to make 20 gallons was added. After 5 minutes' agitation,[2] samples of the settling tests were taken of the mixture in the tank and of the mixture issuing from the nozzle of the spray gun. The spray was then applied.

In test 1, the Bordeaux made with concentrated constituents, and hereafter known as mixture 2, was prepared from the same lots of copper sulphate and lime used above, but instead of diluting one lot, the solution containing 1.6 lb. of copper sulphate per gal. was poured into the suspension containing 1.6 lb. of slaked quicklime per gal. The resulting mixture was stirred thoroughly and poured into the sprayer tank containing the required amount of water to make 20 gallons. After 5 min. agitation, samples for settling tests were taken from the tank and spray nozzle. The spray was then applied.

In tests 2 and 3, mixture 1 was made by slowly pouring 2 lb. of powdered copper sulphate into the sprayer tank containing 18 gal. of water. When the copper sulphate was dissolved, 2 lb. of hydrated lime, thoroughly mixed with 2 gal. of water a few minutes earlier, was poured into the tank while the agitator was running. After agitating 5 minutes, samples for settling tests were taken from spray nozzle and tank, and the spray was then applied.

In tests 2 and 3, mixture 2 was prepared as was mixture 2 in test 1, except that hydrated lime was used. After 5 minutes' agitation, settling-test samples were taken from tank and nozzle. Three trees were then immediately sprayed. The remaining Bordeaux mixture in the tank was agitated for an extra 15 minutes (a total of 35 minutes) and a settling-test sample was taken from the nozzle before it was applied to two other peach trees.

The results of copper analyses are contained in table 1, the results of settling tests for test 1 and 3 are given in figure 1. Settling-test data for test 2 were essentially the same as those for test 3.

Rate of Settling

According to figure 1, mixture 1, taken from the sprayer tank after 5 minutes' agitation, settled very little during 75 minutes, whereas mixture 2

[1] This method is satisfactory for relatively large amounts of copper if the nitric acid is free of nitrous oxide and determinations are made on the wash water immediately after it is filtered.

[2] In this and the other tests the samples of Bordeaux taken from the sprayer tank had not circulated through the pump, whereas that taken from the nozzle of the spray gun had, of course, passed through both pump and nozzle.

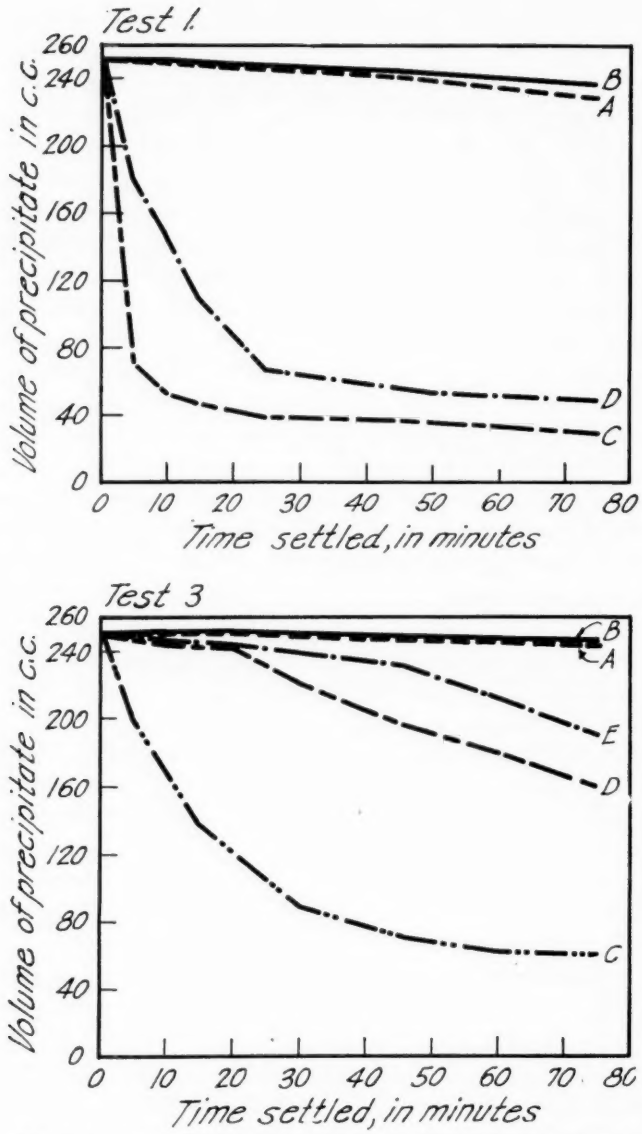

FIG. 1. Effect of method of mixing and the character of agitation on the settling of Bordeaux precipitate. Test 1: A, mixture 1, prepared from diluted lime and concentrated copper sulphate, agitated 5 min. in sprayer tank; B, mixture 1, agitated 5 min. then passed through pump and spray nozzle; C, mixture 2, prepared from concentrated copper sulphate and concentrated lime, agitated 5 min. in sprayer tank; D, mixture 2, agitated 5 min. in sprayer tank, passed through pump and spray nozzle. Test 3: A, mixture 1, prepared from diluted copper sulphate and concentrated lime, agitated 5 min. in sprayer tank; B, mixture 1, agitated 5 min. in sprayer tank then passed through pump and spray nozzle; C, mixture 2, prepared from concentrated copper sulphate and concentrated lime, agitated 5 minutes in sprayer tank; D, mixture 2, agitated 5 minutes in sprayer tank then passed through pump and spray nozzle; E, mixture 2, agitated 35 minutes in sprayer tank then passed through pump and spray nozzle.

from the tank settled very rapidly. In fact, as shown by observations after 18 hours, the latter mixture had settled almost completely within 75 minutes. Mixture 2, which, in addition to 5 minutes' agitation, had gone through the pump and issued from the nozzle of the spray gun, settled somewhat more slowly than that taken from the tank. This was particularly noticeable in test 3. Prolonging agitation of mixture 2 for 35 minutes resulted in a still more slowly settling precipitate.

After settling 18 hours the bulkiness of the precipitate was observed to differ among the Bordeaux mixtures made by the two methods. The volumes (cc.) occupied by the precipitates in test 1 were as follows: Mixture 1 from tank, 72; mixture 1 from nozzle, 103; mixture 2 from tank, 31; mixture 2 from nozzle, 37. With mixture 1 the volume of precipitate was increased by passing through the pump and spray nozzle, whereas mixture 2 was affected little if any.

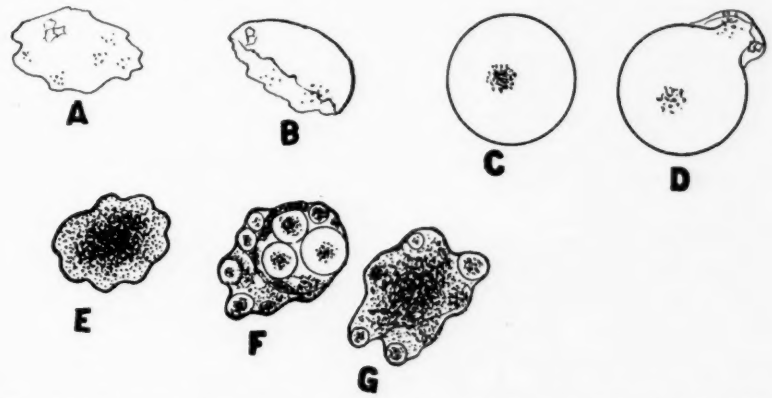

FIG. 2. Characteristics of the particles of precipitate formed when the following concentrations of copper sulphate and lime were mixed: A, limewater and 0.17 per cent copper sulphate; B, both at 0.25 per cent; C–D, both at 0.5, 1, and 2 per cent; E–G, both at 12 per cent.

Judging from the settling tests, the method of diluting one of the constituents (lime in the case of test 1 and copper sulphate in the case of test 3) resulted in precipitates with suspension qualities that could not have been materially excelled by that produced had both constituents been diluted.

To determine what difference existed between the particles of precipitate in mixtures prepared from diluted and concentrated preparations, the following concentrations of $CuSO_4$ solution and lime suspension were mixed with a minimum of agitation: 1. 0.17 per cent lime and 0.168 per cent $CuSO_4$; 2. Both at 0.25 per cent; 3. Both at 0.5 per cent; 4. Both at 1 per cent; 5. Both at 2 per cent, and 6. Both at 12 per cent. Under the microscope the precipitate of No. 1 was composed largely of transparent flakes (Fig. 2, A) that of No. 2 contained a considerable portion of what appeared to be ruptured closed cells (Fig. 2, B). At concentrations of 0.5 to 2 per cent a considerable part of the precipitate was in the form of closed cells

TABLE 1.—*Effect of concentration of the two components, when mixed, on the tenacity of Bordeaux mixture on peach and apricot twigs. December to February 1941–1942*

Test No.[a]	Method of mixing Bordeaux	Tree number and statistical references	Amount of copper on 100 g. of twigs		Copper lost
			Initial deposit	After rain[b]	
			Mg.	*Mg.*	*Per cent*
1	Concentrated copper sulphate poured into diluted lime, agitated 5 minutes before applying. Mixture 1.	1	17.4	15.2	12.6
		2	16.8	12.4	26.2
		3	20.0	16.9	15.5
		Average	**18.0**	**14.8**	**18.1**
	Concentrated copper sulphate poured into concentrated lime, agitated 5 minutes before applying. Mixture 2.	1	22.5	7.8	65.3
		2	19.5	7.3	62.6
		3	21.2	6.8	67.8
		Average	**21.1**	**7.3**	**65.2**
		Diff. for signif.[c]	*9.8*	*3.4*	*20.3*
2	Concentrated lime poured into diluted copper sulphate, agitated 5 minutes before applying. Mixture 1.	1	37.7	24.9	34.0
		2	34.5	24.1	30.1
		3	37.2	23.4	37.1
		Average	**35.7**	**24.1**	**33.7**
	Concentrated copper sulphate poured into concentrated lime, agitated 5 minutes before applying. Mixture 2.	1	26.8	7.7	71.3
		2	31.0	10.2	67.1
		3	29.7	8.0	73.1
		Average	**29.2**	**8.6**	**70.5**
		Diff. for signif.[c]	*5.4*	*2.6*	*12.4*
3	Concentrated lime poured into diluted copper sulphate, agitated 5 minutes before applying. Mixture 1.	1	32.4	21.3	34.3
		2	38.2	23.2	39.3
		3	31.2	19.0	39.1
		Average	**33.9**	**21.2**	**37.6**
	Concentrated copper sulphate poured into concentrated lime, agitated 5 minutes before applying. Mixture 2.	1	19.0	7.6	60.0
		2	22.8	11.8	48.3
		3	26.5	9.5	64.2
		Average	**22.8**	**9.6**	**57.5**
		Diff. for signif.[c]	*5.2*	*3.1*	*23.5*[d]
3A	Concentrated copper sulphate poured into concentrated lime, agitated 35 minutes before applying. Mixture 2.	1	26.9	12.2	54.7
		2	29.2	12.1	58.6
		Average	**28.0**	**12.2**	**56.6**

[a] In test 1 Bordeaux 8–8–100 was prepared with freshly slaked quicklime. In tests 2, 3, and 3A Bordeaux 10–10–100 was prepared with hydrated lime. Trees in tests 1, 3, and 3A were peaches, in test 2, apricots.

[b] The amount of rain falling between application and this analysis was: test 1, 4.12 inches; tests 2, 3, and 3A, 2.96 inches.

[c] The difference for significance calculated on the basis of t values for 99:1 odds.

[d] The difference between means exceed the value for 19:1 odds but not the value for 99:1 odds.

(Fig. 2, C, D). At 2 per cent (No. 5) the membranes surrounding the cells were less transparent than at 0.5 or 1 per cent. At 12 per cent the precipitate (No. 6) was composed of a large number of closed cells illustrated in figure 2, E to G. Some of these cells enclosed dense masses of lime and some enclosed smaller cells along with the lime.

Laboratory tests showed that, whereas such membranes as illustrated in figure 2, C, D, readily broke when the mixture was agitated, and some broke without agitation, those illustrated in 2, E to G did not break so readily. In fact the ordinary whirling motion afforded by a laboratory stirrer broke but few of these particles and had little or no effect on their suspension quality. The increase in suspension, shown above to result from passing mixture 2 through the pump and spray nozzle, may therefore have been because this violent agitation was sufficient to break the surrounding membranes and free the enclosed lime aggregates.

Initial Deposits

In test 1 the initial deposits of the two types of Bordeaux did not differ significantly (Table 1, column 3) but in tests 2 and 3, mixture 1 deposited significantly more copper than mixture 2.

Despite the fact that the analysis showed that mixture 1 deposited as much or more copper than mixture 2 on peach trees, the deposit of the latter was more conspicuous than that of the former. This was because the deposit of mixture 2 was coarser and appeared to stand up from the surfaces, whereas that of mixture 1 was fine in texture and laid closely to the surface. These differences were apparent also when the sprays were deposited on glass slides.

Tenacity of Deposits

In all tests (Table 1) mixture 1 weathered away less rapidly than mixture 2. In test 3 the difference between means of residue after rains and means of percentage of copper lost is significant at 19 : 1 odds but not at 99 : 1 odds. This was largely because the amount of residual copper on tree 2, sprayed with mixture 2, proved exceptionally high.

It will be noted that only 18.1 per cent of the copper in mixture 1 made with quicklime (Test 1) weathered away during rains totaling 4.12 inches, whereas 33.7 and 37.6 per cent, respectively, of that of mixture 1 made with hydrated lime (Tests 2 and 3) weathered away during rains amounting to 2.96 inches. This would suggest that the hydrated lime Bordeaux was less tenacious than quicklime Bordeaux, but should not be considered conclusive proof.

As noted earlier an increase in length of agitation period (Test 3, E) decreased the settling rate of mixture 2, and the deposit studies suggest that there was some increase in tenacity attending this additional agitation, but the data are too meager for conclusions, particularly in view of the large variation between the percentages of copper lost from one tree sprayed with mixture 2 in test 3.

Because uncombined lime gives to a Bordeaux deposit much of its visibility, disappearance of this lime may lead one to believe that the copper deposit also had been lost. Between the two types of deposits studied in these tests, however, such a wide difference in visibility existed after weathering had occurred, that no question arose as to the actual difference in

copper loss. On peach twigs, as noted earlier, the initial deposit of mixture 2, because of its coarse, granular nature, was much more conspicuous than the finer textured initial deposit of mixture 1; but, after weathering, the residue of mixture 2 was visible only around the bases of buds, whereas that of mixture 1 remained visible over all the twig surface.

DISCUSSION

The results just given substantiate the general belief that the physical characteristics of Bordeaux precipitate, which are determined by the concentration of the copper sulphate and lime preparations, play an important role in determining the efficacy of this fungicide. The criterion of efficacy in these studies was the resistance of the copper deposit to washing off by rain (tenacity). The precautions against mixing concentrated, *i.e.*, 12 per cent, copper sulphate and lime, therefore, appear justified when maximum tenacity to such twig surfaces as those of peaches and apricots is desired.

Settling tests almost always show Bordeaux mixture, prepared by combining concentrated components, possesses poor suspension quality. Rapid settling can be decreased to some extent by agitation, particularly by passage of the mixture through the pump and nozzle, but, within the limits of practicability, it is doubtful if agitation can entirely overcome this feature. Agitation probably breaks the closed cells and disperses lime aggregates, but whether it has any effect on the nature of the membranes themselves is not known. The results of one test, suggesting that the tenacity of Bordeaux made with concentrated constituents was improved somewhat by prolonged agitation, are interesting but not conclusive.

The differences in texture of precipitates apparent, in the dried deposits of the two types of Bordeaux mixtures, suggests one reason for the low tenacity of the deposit of that made with concentrated, constituents. Being coarse, this deposit stands up from the surface and probably is more exposed to erosion by rain than the finer deposit of Bordeaux made with diluted constituents.

Effects of differences in mixing on the depositional qualities of the resulting mixtures were not conclusively proved, though in two of the three tests Bordeaux mixture prepared with diluted components deposited significantly more copper than Bordeaux mixture prepared with concentrated components. To thoroughly explore this subject, retention of the liquid phase and behavior of the solid phase during application should be studied carefully.

SUMMARY AND CONCLUSIONS

Bordeaux precipitate made by mixing diluted components was settled more slowly and was more bulky than that made by mixing concentrated components (12 per cent) and then diluting. The individual particles of the precipitate of the former (components diluted) were nearly all fragments of closed cells that ruptured during the reaction between the components or

during the moderate agitation given in laboratory tests. The membranous fragments were thin, pliable, and possibly at a high state of hydration. The particles of the latter (components concentrated) were in many cases agglomerations of lime held together by closed membranes that did not easily rupture during the reaction and apparently were not easily broken by agitation.

In 3 weathering tests conducted on twigs of peach and apricot trees during the winter, when rainfall totaled 3 to 4 inches, Bordeaux, prepared with diluted components, lost only 18, 34, and 38 per cent of the copper, whereas the Bordeaux prepared with concentrated components lost 65, 70, and 58 per cent.

Vigorous and prolonged agitation improved the suspension quality of the concentrated type. One test suggested that the agitation possibly improved its weathering quality, but the differences were not great enough for statistical significance.

In two of three tests the Bordeaux made with diluted constituents deposited sigificantly more copper than that made with concentrated constituents. The initial deposit of the latter, however, was more visible on peach twigs because it was coarser in texture and stood up from the surface more than that of the former.

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TESTS OF ERADICANT SPRAYS FOR USE AGAINST SCLEROTINIA LAXA AND CORYNEUM BEIJERINCKII IN APRICOTS AND ALMONDS

E. E. Wilson

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Two fungus diseases, the brown-rot blossom and twig blight (*Sclerotinia laxa* Ader. and Ruh.) and the shot-hole (*Coryneum baijerinckii* Oud.), are widespread and destructive in apricot and almond orchards in California. Brown-rot blossom blight recently was shown[1] to be measurably reduced by a dormant application of calcium arsenite[2] (2 or 3 lb. per 100 gal. of water). The object of spraying with this material is to prevent the production of the conidia-bearing mats (sporodochia) on blighted, hold-over twigs in the tree and to destroy the sporodochia already present. So far as known these blighted twigs and the occasional rotted fruits that hang in the tree are the only sources of inoculum for blossom infection in the spring. The mycelium of the fungus, surviving in these parts from one season to the next, develops the sporodochia, which break through to the surface and produce conidia in late winter. Shot hole affects buds of apricot (Fig. 1), the buds and twigs of almond in winter, and blossoms, leaves, and fruit of both in spring. Apparently, only conidia are produced by the causal fungus in California.[3] These are found in greater or less abundance on the various diseased organs, but the mycelium in diseased buds, twigs, and an occasional blighted blossom remaining in the tree, is the only known means whereby the fungus survives from season to season. Conidia form the year around within blighted buds, but apparently only in winter and spring on the surface of twig lesions. The problem of controlling this disease by eradicant means, therefore, is that of preventing the fungus from surviving or from producing spores in these host parts.

The calcium arsenite spray is being used in California for control of *Sclerotinia laxa* and with comparatively little injury to apricot trees. It cannot, however, be safely applied to almond and certain prune varieties. An alternate material, harmless to the trees and effective against both *S. laxa* and *Coryneum beijerinckii*, would, therefore, be of great value. The writer here reports tests in which a number of compounds were tested against both fungi.

EFFECT OF VARIOUS MATERIALS ON PRODUCTION OF SPORODOCHIA OF SCLEROTINIA LAXA ON BLIGHTED TWIGS

Sodium dinitro-o-cresylate (Elgetol containing 30 per cent of this salt) was included in tests with calcium arsenite as early as 1939. In table 1 are

[1] Wilson, E. E. Experiments with arsenite sprays to eradicate *Sclerotinia laxa* in stone-fruit trees as a means of controlling the brown rot disease in blossoms. Jour. Agr. Res. [U.S.] **64**: 561–594. 1942.

[2] Although called monocalcium arsenite in the article cited above, there are reasons for believing this is not the proper designation. In any event, the material is one of the less basic of the calcium arsenites.

[3] A recent search in infected, overwintered leaves and in diseased buds and twigs of almond revealed no other stage.

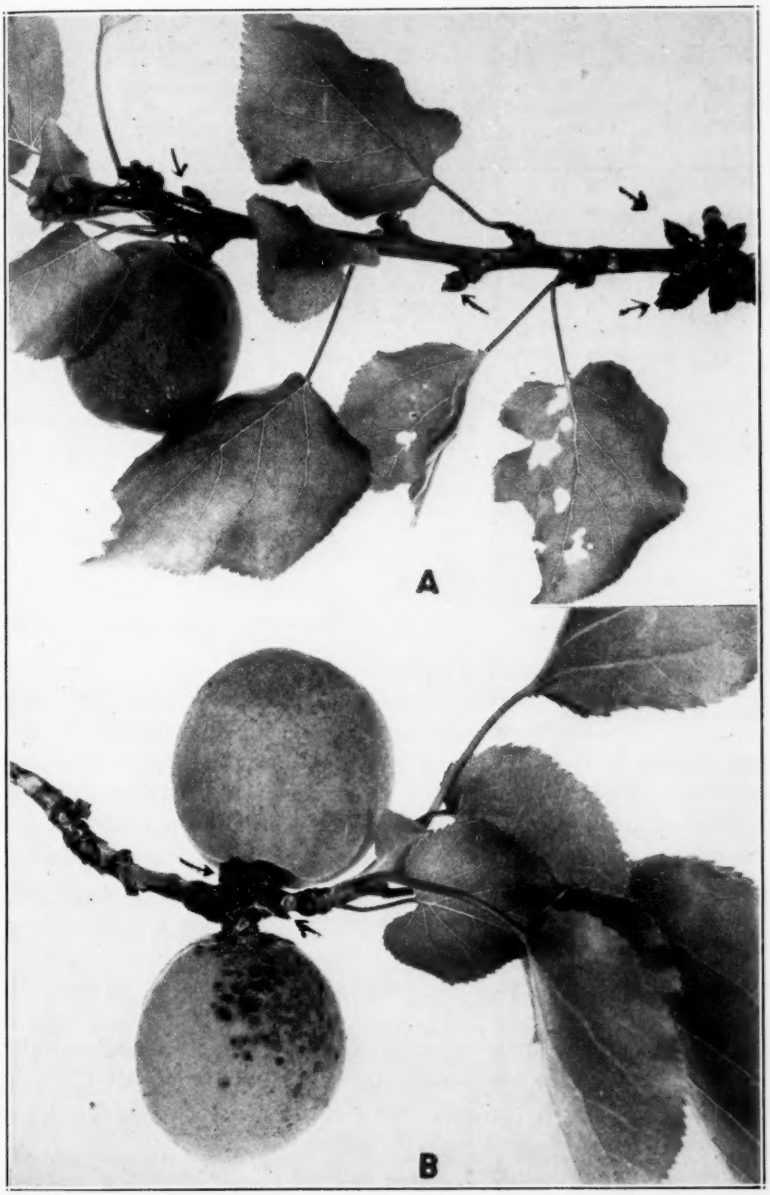

FIG. 1. Shot hole (*Coryneum beijerinckii*) affecting buds and fruit of apricot. A. Blighted buds (arrows) furnish conidia that infect leaves (infected leaves with holes) and fruit in spring. B. Fruit borne above blighted buds, healthy, whereas that below such buds, badly affected.

TABLE 1.—*Effect of spraying with dinitro-o-cresylates on the production of sporodochia by Sclerotinia laxa in apricot and almond trees during the winter*

Kind of tree and year	Materials used and date of spraying	Reduction in the abundance of sporodochia on holdover twigs[a]	Reduction in the infection of blossoming shoots
		Per cent	*Per cent*
Apricot, 1939	Sodium dinitro-o-cresylate (Elgetol), 0.3 per cent, Jan. 18	95	78
Almond, 1939	Sodium dinitro-o-cresylate, 0.3 per cent, Jan. 17	81	12
Prune, 1939	Sodium dinitro-o-cresylate, 0.3 per cent, Dec. 15	53	.....
Almond, 1940	Sodium dinitro-o-cresylate, 0.3 per cent, Feb. 1	92	68
Apricot, 1941	Sodium dinitro-o-cresylate, 0.3 per cent, Dec. 9	48	.....
	Sodium dinitro-o-cresylate, 0.3 per cent, plus ammonium sulphate, 0.5 per cent, Dec. 9[b]	41	.....
Apricot, 1941	Sodium dinitro-o-cresylate, 0.3 per cent, Feb. 4, 1941	80	.....
	Triethanolamine dinitro-o-cresylate, 0.3 per cent, Feb. 4	65	.....
Almond, 1942	Sodium dinitro-o-cresylate, 0.3 per cent, Jan. 7	56	2

[a] Data taken at the end of bloom period.

[b] Ammonium dinitro-o-cresylate was presumably produced by adding ammonium sulphate to the sodium salt.

included all tests in which the object was to observe the effect of this material on production of the sporodochia by *Sclerotinia laxa*. These data show a great variation in the percentage reduction of sporodochia,[4] when the material was used at a strength of 0.3 per cent of the salt. A reduction of 92 and 95 per cent., respectively, accompanied its used in two tests, 80 and 81 per cent in two tests and 48, 53, and 56 per cent in three tests. A decrease in blossom blighting followed the reduction in sporodochial development, but was marked only where a high degree of suppression of sporodochia was obtained.

In one instance where ammonium sulphate was added to sodium dinitro-o-cresylate, thus producing the ammonium salt of dinitro-o-cresol, no difference in results followed. In one test triethanolamine dinitro-o-cresylate proved somewhat less effective than the sodium salt.

Furthermore, according to the data in figure 2, the suppressive effect of sodium dinitro-o-cresylate decreased between the beginning and the end of bloom. In 1939, for example, reduction in the number of sporodochia at the beginning of bloom was 93 per cent, at the end of bloom 53 per cent. In 1941 the percentages were 92 and 80, respectively, and in 1942, 80 and 56.

[4] The percentage of the diseased, holdover twigs that produced sporodochia and the average number of sporodochia per twig were determined on 200 randomly selected twigs from each treated and nontreated plot. The product of these two values provided index numbers from which the percentage reduction of sporodochia by the sprays was calculated.

These data, furthermore, indicate, as did those in table 1, that (with one exception in table 1) the suppression induced by February applications was greater than that following the December and January applications. In order to test this relationship further, small-scale applications on January 9 1942, were compared with those made on February 2, 1942. At the earlier date sporodochia had not appeared on the twigs, nor was there evidence that they were breaking through the twig periderm. At the latter date, however, sporodochia with conidia had appeared, and numerous others were breaking through the periderm. When applied at the time sporodochia were becoming exposed on the twig surfaces, the spray reduced their development by 77 per cent, but when applied before the sporodochia became exposed the reduction was only 39 per cent.

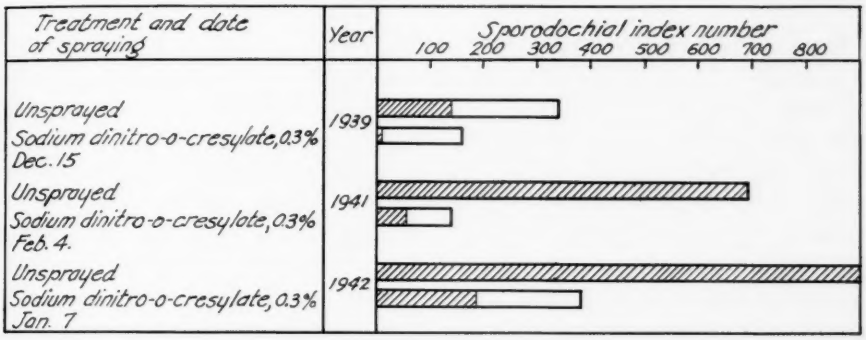

FIG. 2. Relative abundance of sporodochia at the beginning (cross bar) and at the end (cross bar plus white bar) of blossoming period, in nonsprayed apricot and almond trees and in similar trees sprayed in winter and in early spring with sodium dinitro-o-cresylate. The sporodochial index number is the product of the percentage of twigs with sporodochia and the average number of sporodochia per twig.

To determine the effect of the spray on the viability of the fungus within the tissues, twigs receiving sodium dinitro-o-cresylate and nontreated twigs were cultured. The number of sprayed and nonsprayed twigs with live mycelium differed but little, showing that the spray was not, under the conditions of these tests, killing the mycelium of the fungus embedded in host tissue. Apparently, therefore, the failure of the spray to kill embedded mycelium accounts for the development of new sporodochia between the beginning and end of bloom.

The suppressive effect of other materials also was tested in field studies between 1939 and 1942. These were tar oil-emulsion, 3 per cent; potassium ethyl xanthate, 1 per cent; sodium dimethyl-dithio-carbamate, 0.5 per cent; ferric dimethyl-dithio-carbamate, 0.5 per cent; an emulsible cresol, 1 per cent; zinc sulphate, 0.5 per cent; and ammonium sulphate, 0.5 per cent. None of these measurably reduced the development of sporodochia. In 2 tests of copper aceto-arsenite (Paris green) 0.5 per cent satisfactory suppression resulted, but in one test the results were unsatisfactory. This

material was objectionable in that it seriously injured almond trees in the 2 tests where suppression of sporodochia was most satisfactory.

EFFECT OF VARIOUS MATERIALS ON SPORODOCHIA OF SCLEROTINIA LAXA

Attention was next directed to the effect of delaying the sprays until shortly before the blossoming period, when most of the sporodochia are exposed on the twig surfaces. If the spray destroyed most of the conidia on these structures, blossom infection might be lowered provided the mycelium in the twigs produced no new conidia before the trees were through blooming. Spraying cannot, however, be delayed much after the blossom buds begin to break, since the materials are known to injure the blossoms. Hence, in some seasons as much as two weeks will elapse between spraying and the beginning of bloom.

In order to test more materials, and replicate tests of the more promising ones, the following method was employed: Blighted, hold-over almond and apricot twigs bearing sporodochia were cut from the tree and stood upright in moist sand in 6-inch flower pots. They were then thoroughly sprayed with the desired material and after the spray dried were placed in a lath house where the conditions were essentially those of the outdoors except that strong winds were avoided. After a period of one week the spores from 10 to 15 sporodochia, picked at random from each group of sprayed and nonsprayed twigs, were suspended in separate lots of sterile, distilled water. The number of spores in each suspension was adjusted to about the same value by the addition of water, and drops of these suspensions were placed on the surface of each of several freshly poured plates of potato-dextrose agar. After 15 hours incubation at 25° C., the percentage germination of spores was determined. The conditions under which these experiments were conducted favored development of new sporodochia and conidia; so, by holding the twigs for a week after the germination tests, the effect of the materials on further development of these structures also was determined.

Forty-one materials were tested in 3 tests in February and March, 1941. The following at 1 per cent concentration neither destroyed the conidia nor prevented their further production: Paris green; ferric, sodium, and copper dimethyl-dithio-carbamates; sodium, copper, and lauryl thiocyanates; copper, zinc, and ammonium sulphates; an emulsible cresol; mercurochrome; salicylic acid; oxalic acid; hydroquinone; emulsions of paraformaldehyde, xylol, and paradichlorobenzene; an alcoholic solution of iodine; phenothiazine; calcium cresylate; calcium cyanamid; sodium chlorate; potassium permanganate; potassium dichromate; mercurous chloride; emulsified pine oil; and emulsified furfural. The following also were ineffective: 10 per cent lime-sulphur, 2 per cent tar oil-emulsion, 0.4 per cent ethyl mercury phosphate (Lignasan), and 0.25 per cent arsenic trioxide.

Six materials showed enough promise to be included in further trials. One per cent sodium tetrachloro-phenate, 1 per cent sodium pentachloro-

phenate, 0.45 per cent sodium dinitro-o-cresylate and 1 per cent sodium orthophenyl-phenate reduced germinability of conidia 96, 80, 73, and 58 per cent, respectively. One per cent sodium tetrachloro-p-benzoquinone dissolved in a 0.32 per cent sodium hydroxide solution reduced germinability 47 per cent, whereas 1 per cent 4-chloro-1, 2-benzoquinone dioxime dissolved in 1 per cent ammonium hydroxide reduced germinability only 39 per cent. None of these materials, however, prevented the production of new conidia. Apparently, therefore, none penetrated the twigs sufficiently to reach and kill the mycelium therein.

In early February, 1942, before resumption of tree growth but after sporodochia became abundant on hold-over twigs, the following materials were applied in one apricot and two almond orchards: 0.5, 0.3, and 0.15 per cent solutions of sodium dinitro-o-cresylate; 0.15 per cent emulsion of dinitro-o-cresol; 0.37 and 0.5 per cent solutions of sodium orthophenyl phenate, sodium tetrachloro-phenate, and sodium pentachloro-phenate; 0.15 per cent of orthophenyl-, tetrachloro-, and pentachloro-phenols, which were dissolved in petroleum oil; and 0.5 per cent tetrachloro-p-benzoquinone dissolved in a 0.3 per cent aqueous solution of sodium hydroxide. About 2 to 3 weeks after spraying, when the trees began to bloom, the conidia in sprayed and nonsprayed trees were tested for germinability.

According to the results from these tests the highest reductions in germinability were 53 and 55 per cent, given by 0.5 per cent sodium dinitro-o-cresylate and 0.37 per cent sodium tetrachloro-phenate, respectively; the next highest was 44 per cent given by pentachloro-phenol in oil. This low reduction in the abundance of viable inoculum was reflected in one orchard where the disease developed. In this orchard the 53 per cent reduction in viable conidia by sodium dinito-o-cresylate was followed by only a 12 per cent reduction of blossom blighting.

As the eradicant effects of the materials in field tests were much less than their effects in the lath house tests, an effort was made to determine the reason. One reason seemed to be the failure of the materials to penetrate into the mass of conidia as they occur on the sporodochia. In two of the field trials a wetting agent was added. This material previously had been shown to increase the penetration of conidial masses by dinitro-o-cresylates and chloro-phenates. Despite the addition of this material, however, the spores at the center of the sporodochia were unaffected by the sprays, though the spores at the periphery were largely killed.

Possibly another reason for the inconsistency between lath-house and field results is the high solubility of the sodium salts of dinitro-o-cresylate, and the chloro-phenate. If, as happened in some of the field tests, heavy rains occur a few days after the sprays are applied, the materials may be washed from the tree before they exert their maximum influence against the fungus. Where twigs sprayed with sodium dinitro-o-cresylate were kept moist but were protected from rains by a bell jar, the percentage of spores killed within 5 days was ordinarily much higher than where twigs were exposed to rains.

EFFECT OF SODIUM DINITRO-O-CRESYLATE, SODIUM ORTHOPHENYL-PHENATE, AND THE SODIUM CHLORO-PHENATES ON CONIDIA AND MYCELIUM OF CORYNEUM BEIJERINCKII

Calcium arsenite applied in January was found to reduce the incidence of the infection of almond leaves by *Coryneum beijerinckii* in the spring. In most cases the reduction was noticeable only where the trees had been sprayed with 4 pounds of calcium arsenite to 100 gallons of water. In one instance this concentration killed 75 per cent of the conidia of *C. beijerinckii* within blighted apricot buds. Concentration of 2 and 3 pounds to 100 gallons of water, on the other hand, failed to satisfactorily reduce apricot fruit infection by this fungus. As a concentration of 4–100 is too injurious to use on apricot trees, which are even more tolerant of arsenites than almonds, calcium arsenite apparently is not a satisfactory eradicant against this fungus.

In order to determine if the benzene derivatives were effective against *Coryneum beijerinckii,* preliminary tests were carried out in the lath house. Twigs of apricot and peach, bearing dormant buds blighted by the fungus, were collected in the winter. These were stood upright in moist sand and sprayed with various concentrations of the test materials. After drying the twigs were held in the lath house for one week. In all tests rains fell during this period.

According to the data in figure 3 all materials were noticeably lethal to the conidia of *Coryneum beijerinckii* borne on the inside of the diseased dormant buds. The most effective were sodium dinitro-o-cresylate, triethanolamine dinitro-o-cresylate, sodium pentachloro-phenate, and sodium tetrachloro-phenate. Somewhat less effective were 4-chloro-1, 2-benzoquinone, dioxime, tetrachloro-p-benzoquinone, and sodium trichloro-phenate. The least effective was sodium orthophenyl-phenate. Most of the materials appeared to be equally effective at 0.5 and 1 per cent, but all were less effective at 0.25 per cent.

In other tests, twigs sprayed with sodium dinitro-o-cresylate and the chloro-phenates were kept in moist chambers to prevent the sprays from drying. Within 48 hours most of the conidia inside the diseased buds were badly shriveled.

Sodium dinitro-o-cresylate, 0.5 and 0.3 per cent, applied to almond trees on January 29 did not materially reduce the disease subsequently developing on fruit and leaves, although an examination at the beginning of bloom showed that but few viable condia were present in blighted buds and on the surface of blighted spurs. Judging from the results given in table 2 and discussed below, it seems likely that between the time this examination was made and the development of leaves and fruit the fungus might have produced a new lot of conidia in buds on the treated trees.

The effect of sodium tetrachloro-phenate, sodium pentachloro-phenate, and tetrachloro-p-benzoquinone on conidia in apricot buds is shown in table

2. The sprays were applied on February 11 as the blossom buds were swelling. Examinations at the beginning (Feb. 18) and at the end of bloom (March 3) showed few viable conidia in buds sprayed with the chlorophenates, but somewhat more in those sprayed with the benzoquinone. On May 14, however, after the disease had made its appearance on fruit there

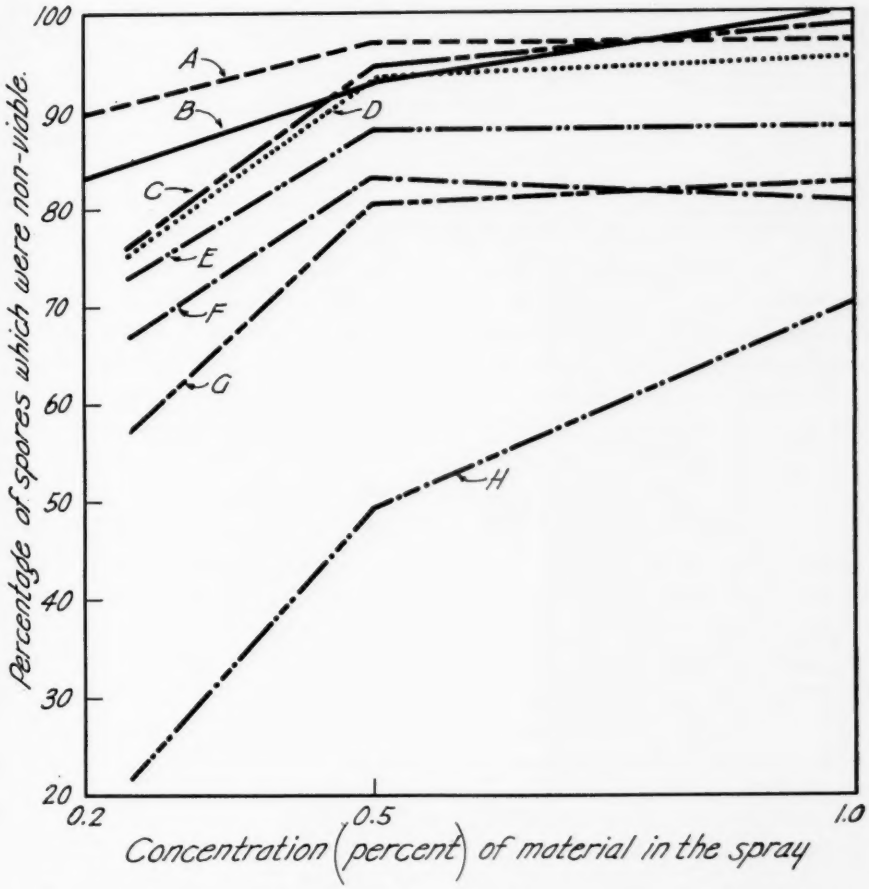

FIG. 3. Effect of spraying with different materials on the conidia of *Coryneum beijerinckii* in the diseased peach buds. A. Sodium dinitro-o-cresylate. B. Triethanolamine dinitro-o-cresylate. C. Sodium pentachloro-phenate. D. Sodium tetrachloro-phenate. E. 4-chloro-1, 2-benzoquinone dioxime dissolved in ammonium hydroxide. F. Sodium trichloro-phenate. G. Tetrachloro-p-benzoquinone dissolved in sodium hydroxide. H. Sodium orthophenyl-phenate.

were numerous viable conidia in buds sprayed with all materials. Leaf infection was too sparse for test purposes, but fruit infection was somewhat more abundant. The data (Table 2, column 5) show that none of the materials significantly reduced the disease on the fruit.

DISCUSSION

Since Keitt[5] described the eradicant effect of sodium dinitro-o-cresylate (Elgetol) against the perithecial stage of *Venturia inaequalis* (Cke.) Wint. in over-wintered apple leaves, a number of apparently successful attempts to control this and other diseases have been reported. So far as the

TABLE 2.—*Effect of spraying with the chloro-phenates and tetrachloro-p-benzoquinone on the conidia of Coryneum beijerinckii in diseased buds and on apricot fruit infection, 1942*

Treatment on February 11[a]	Percentage spores nonviable when taken from buds on			Percentage fruit infected on May 14
	Feb. 18[b]	March 3[c]	May 14[d]	
Nonsprayed	15	21	27	17
Sodium tetrachloro-phenate, 0.5 per cent	84	97	39	12
Sodium pentachloro-phenate, 0.5 per cent	90	96	33	14
Tetrachloro-p-benzoquinone, 0.5 per cent[e]	57	89	21	18
Difference for significance at 99:1 odds	11	5	9	6

[a] An organic wetting agent, 0.1 per cent added to each spray.
[b] Blossoms opening.
[c] Fruit beginning to grow.
[d] Fruit ⅓ grown.
[e] To dissolve this material 5 oz. of sodium hydroxide was added for each pound.

present writer is aware, however, no tests of other cresylates, of the phenates, or of related compounds have been reported. Although, on the whole, unsuccessful as eradicants against the fungi tested herein, the phenates, particularly tetrachloro- and pentachloro-phenate, show enough promise to be tried against such fungi as have yielded to sodium dinitro-o-cresylate.

None of these materials approached the effectiveness of calcium arsenite in suppressing the development of sporodochia by *Sclerotinia laxa*. Partial suppression is little better than no suppression, since the fungus produces such an abundance of sporodochia, especially in almond trees. The relation of the degree of sporodochial suppression to the degree of reduction in blossom infection is illustrated in table 1, where a 95 per cent reduction of sporodochial development in 1939 was accompanied by a 78 per cent decrease in blossom blighting, whereas an 81 per cent reduction in the same year was accompanied by only a 12 per cent decrease.

One weakness of these materials as eradicants against both *Sclerotinia laxa* and *Coryneum beijerinckii* is their failure to kill the mycelium within the twigs and buds. A second point of weakness is their failure to penetrate the masses of conidia produced by *Sclerotinia laxa* on the sporodochia. It may be that their penetration into the sporodochia will be increased by a

[5] Keitt, G. W. Toxicity of the sodium salt of dinitro-o-cresol to *Venturia inaequalis*. Science (n. s.) **90**: 139–140. 1939.

proper supplementary material added to the spray, but such a material is yet to be found. A third point of weakness is believed to be their solubility. How much can be done with less soluble salts is yet to be shown.

To a certain extent failure of sodium dinitro-o-cresylate to lower the incidence of the shot hole on almond leaves and fruit is attributable, possibly, to occurrence of secondary infection. With this disease the critical period for attack of leaves and fruit extends from the time they appear until spring rains of sufficient length to allow for infection are at an end. The fungus produces conidia in abundance on infected blossoms and very young leaves, hence each one is a source of conidia for secondary infection.

SUMMARY AND CONCLUSIONS

Of 41 materials tested for ability to eradicate the holdover stage of *Sclerotinia laxa* from apricot and almond trees, only 6 showed enough promise for further tests. These were 0.5 and 0.3 per cent solutions of sodium dinitro-o-cresylate (Elgetol), and 1 per cent solutions of sodium tetrachloro-phenate, sodium pentachloro-phenate, sodium orthophenyl-phenate, tetrachloro-p-benzoquinone, and 4-chloro-1, 2-benzoquinone dioxime.

In extended trials these materials showed only moderate ability to prevent the sporodochia (the conidia-bearing mats) from developing on the blighted twigs during the winter primarily because they did not kill the mycelium of the fungus within these twigs.

After sporodochia developed, however, the materials under some conditions were fairly effective in destroying them and the conidia borne thereon. Sodium dinitro-o-cresylate, sodium tetrachloro-phenate, and sodium pentachloro-phenate proved somewhat more injurious to the sporodochial stage than sodium orthophenyl-phenate, tetrachloro-p-benzoquinone or 4-chloro-1, 2-benzoquinone dioxime. Wide variability in the percentage of conidia killed, however, was experienced in different tests. In orchard tests in 1942 none reduced the primary inoculum sufficiently to insure an appreciable lowering of the amount of blossom infection by the fungus.

Triethanolamine dinitro-o-cresylate, ammonium dinitro-o-cresylate, dinitro-o-cresol, orthophenyl-phenol, tetrachloro-phenol, and pentachloro-phenol, at the concentrations used, proved no more effective than the sodium salts.

The sodium salts of dinitro-o-cresol and the two chloro-phenols were about equally effective in killing the conidia of *Coryneum beijerinckii*, which are produced within blighted dormant buds on apricot, peach, and almond and constitute the primary inoculum for fruit and leaf infection in the spring. Within one week after applying 0.5 or 1 per cent solutions of these materials to diseased twigs, 80 to 95 per cent of the conidia were dead. Orchard tests, however, proved that the fungus later produced numerous new condia within sprayed buds, apparently because the spray had not killed the mycelium within the host tissue. Unsatisfactory control of fruit

and leaf infection in almond and apricot resulted, both because of this production of new conidia and possibly because of secondary infection arising from conidia produced on leaves and blossoms that were infected by primary inoculum from the buds.

Unless these materials are rendered more capable of penetrating the conidial masses on the sporodochia of *Sclerotinia laxa* and of entering the tissues and killing the mycelium both of *S. laxa* and *Coryneum beijerinckii* in the diseased twigs, they apparently will not be successful as eradicant sprays against the diseases in question.

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EFFECTS OF EXTRACT OF WESTERN RED-CEDAR HEARTWOOD ON CERTAIN WOOD-DECAYING FUNGI IN CULTURE[1]

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INTRODUCTION

Previous workers have shown that the water-soluble extractives of western red-cedar (*Thuja plicata* D. Don) heartwood include a substance, probably phenolic, which is toxic to *Lenzites saepiaria* Wulf. ex Fr., *Lentinus lepideus* Fr., *Fomes annosus* (Fr.) Cooke, and "Madison 517"[4] (4, 7, 1, 2). Removal of this substance by water leaching results in a marked reduction in the well-known resistance of the heartwood to fungal decay (4, 7, 5, 6). In spite of the natural durability of western red-cedar heartwood in service, heart rot is common in mature stands. Since fungi evidently invade the heartwood in spite of the toxic substance, it seemed desirable to investigate the effects of various concentrations of western red-cedar heartwood extract on growth of certain common wood-decaying fungi in culture.

EXPERIMENTAL WORK

Preparation of Extract and Media

The extract used was prepared by reducing to sawdust the heartwood of a bolt cut from the lower part of the trunk of a freshly felled western red-cedar approximately 15 inches in diameter at breast height (4.5 feet). A 950-g. portion of this sawdust was placed in flasks and covered with distilled water. The mixture was kept, with occasional shaking, for a period of two weeks, during which time it was maintained at a temperature of 100° C. (by means of flowing steam) for a total of 50 hours. No attempt was made to avoid oxidation of the extractives. Upon filtering through a Buchner funnel, 8 l. of filtrate were obtained. Since 8000 ml. of filtrate were recovered from 950 g. of wood, 8.4 ml. contained the extract from 1 g. of wood. A 1-per cent solution (defined as a solution containing the extract from 1 g. of wood in 100 ml. of solution) was prepared by taking 8.4 ml. of the above extract and making up to 100 ml. with distilled water. This system was employed in order to provide a standard method of designating concentrations of extracts based on the weight of wood represented per unit volume of extract. When the results of the present experiments are compared with those of a preliminary experiment, in which an-

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[4] "Madison 517" is culture number 517 of the Forest Products Laboratory, Madison, Wisconsin. This culture is the same one used by Anderson and Sherrard (1), who referred to it as *Fomes annosus* (personal communication from E. C. Sherrard). Cartwright (in Trans. Brit. Mye. Soc. 22: 232–233. 1939) has shown that it is probably *Polyporus tulipiferus*.

other batch of extract was used, and with those of other workers (7, 2) after converting their figures into terms of percentage, it becomes evident that such percentage figures denote rather definite degrees of toxicity, although it is realized that the degree of toxicity for a given concentration of extract will vary with the concentration of toxic substances in the wood sample and with the completeness of the extraction.

Media containing the toxic extract were made according to the following formula:

2 g. "Difco Bacto-agar"
2.5 g. desiccated "Difco malt extract"
Volume of aqueous heartwood extract necessary to furnish desired percentage
Volume of water to make up to 100 ml.

In order to prepare media containing over 10 per cent of extract it was necessary to concentrate the extract. For this purpose some of the extract was evaporated so that 4 ml. represented 1 g. of wood. The evaporation was carried on at 50° C., at atmospheric pressure.

Manipulation of Fungi

The following fungi were used in the tests: *Coniophora puteana* (Schum. ex Fr.) Karst. = *C. cerebella* (Pers. ex Fr.) Schroet., *Fomes officinalis* (Vill. ex. Fr.) Faull = *F. laricis* (Jacq. ex Fr.) Murr., *F. pinicola* (Swartz) Cooke, *F. roseus* (Alb. and Schw. ex Fr.) Cooke, *Lentinus lepideus* Fr., "Madison 517," *Polyporus schweinitzii* Fr., *P. sulphureus* Bull. ex Fr., *Poria xantha* (Fr.) Lind. forma *crassa* (Karst.) Baxt., *Trametes serialis* Fr., and *T. subrosea* Weir.

Circular planting pieces 1 sq. cm. in area were transferred, mycelium side up, from the margins of actively growing malt-agar, Petri-dish cultures to the centers of duplicate Petri dishes of test media containing various concentrations of western red-cedar heartwood extract. Duplicate control plantings were made on medium prepared as described above but without any extract. The diameter of the resulting mycelial mats was measured every 4 days until the mycelium on the control medium reached the edges of the plates. Growth was recorded as percentage of normal growth (diameter of fungus on test medium $\times$ 100 / diameter of fungus on control medium).

Results of Growth Tests

Since measurements of duplicate cultures were found to be practically identical (with a few exceptions on media containing high concentrations of extract), only the means of measurements in duplicate plates are presented. Table 1 shows that any considerable concentration of the extract (4 per cent or over) retarded the rate of growth of all of the tested fungi, and that somewhat higher concentrations completely prevented growth. This is in agreement with the findings of other workers (4, 7, 1, 2, 3).

TABLE 1.—*Percentage of normal growth on malt agar containing various concentrations of western red-cedar heartwood extract*

Species tested[a]	Age of culture	Percentage concentration of extract in medium[b]													
		¼	½	1	2	4	6	8	10	12	14	16	18	20	22	24
	Days	*Per cent of normal growth*[c]														
Trametes serialis	4	100	100	90	85	60	40	5	G	G	G	G	0	0	0	0
	8	100	95	95	82	60	45	35	35	40	35	30	G	F	0	D
Fomes roseus	4	100	110	110	110	92	60	G	0	0	0	0	0	0	0	0
	8	100	105	108	95	90	70	53	50	40	37	0	0	0	0	0
	12	100	100	100	95	90	80	65	63	60	50	F	F	F	0	D
Trametes subrosea	4	110	100	95	90	60	G	0	0	0	0	0	0	0	0	0
	8	107	105	100	100	80	60	40	30	30	33	0	0	0	0	0
	12	105	100	100	100	90	83	60	45	37	40	0	0	0	0	D
"Madison 517"	4	100	100	100	90	75	60	45	25	28	24	0	0	0	0	0
	8	100	95	90	90	85	73	60	30	30	25	F	F	F	0	D
Lentinus lepideus	4	100	100	100	100	80	60	G	G	G	0	0	0	0	0	0
	8	100	105	110	95	83	70	50	45	30	0	0	0	0	0	0
	12	110	100	100	90	80	75	60	55	35	F	F	F	F	0	D
Polyporus sulphureus	4	100	105	105	100	55	G	0	0	0	0	0	0	0	0	0
	8	100	105	105	100	80	63	40	32	32	D	D	D	D	D	D
Fomes officinalis	4	H	H	0	0	0	0	0	0	0	0	0	0	0	0	0
	8	150	177	G	G	G	0	0	0	0	0	0	0	0	0	0
	12	125	134	65	G	G	0	0	0	0	0	0	0	0	0	0
	16	120	125	81	71	64	20	0	0	0	0	0	0	0	0	0
	20	115	113	90	80	80	40	G	F	F	F	F	F	F	F	F[d]
Polyporus schweinitzii	4	100	105	100	50	0	0	0	0	0	0	0	0	0	0	0
	8	100	100	100	70	F	F	F	F	F	D	D	D	D	D	D
Fomes pinicola	4	100	90	80	60	G	0	0	0	0	0	0	0	0	0	0
	8	95	85	77	62	42	G	0	0	0	0	0	0	0	0	0
	12	100	85	78	70	48	20	D	D	D	D	D	D	D	D	D
Coniophora puteana	4	100	100	100	83	G	0	0	0	0	0	0	0	0	0	0
	8	100	100	100	90	54	0	0	0	0	0	0	0	0	0	0
	12	100	100	100	95	58	D	D	D	D[e]	D	D	D	D	D	D
Poria xantha f. *crassa*	4	105	120	95	G	0	0	0	0	0	0	0	0	0	0	0
	8	130	130	110	10	G	0	0	0	0	0	0	0	0	0	0
	12	110	110	105	88	55	D	D	D	D	D	D	D	D	D	D

G = growth evident on planting piece but not on test medium.
H = hormesis: stimulation indicated by growth on test medium prior to growth on control medium.
F = fungistasis: growth prevented but mycelium not killed.
D = death: mycelium failed to grow when planting piece transferred to normal medium.

[a] Fungi arranged in order of decreasing growth.
[b] Grams of wood represented per 100 ml. of medium.
[c] "Normal growth" refers to growth on control medium.
[d] Another batch of extract was found to be lethal to *Fomes officinalis*. Growth rates of other fungi on various dilutions of this extract indicate that its concentration was probably between 30 and 35 per cent.
[e] In another series of tests one planting piece of *Coniophora puteana* grew on test medium containing 12 per cent of extract.

In order to learn whether the planting pieces that did not grow were dead, or merely static, they were removed to ordinary malt-agar medium after the test period. This test showed that all non-growing planting pieces of *Coniophora puteana*, *Fomes pinicola*, *Polyporus sulphureus*, *Poria xantha* f. *crassa*, and *Trametes subrosea* were dead, whereas some planting pieces of *Fomes officinalis*, *F. roseus*, *Lentinus lepideus*, "Madison 517," and *Poly-*

porus schweinitzii resumed growth. These results indicate that within the test period some concentrations of the toxic extract were completely fungistatic, but not lethal, to certain of the fungi.[5] This study did not indicate whether continued exposure would kill the static plantings, or whether they would eventually grow.

An increase in growth rate of many of the fungi in extreme dilutions of the extract is also evident from the data in table 1. This accelerated growth rate tended to decrease with time and approach the normal rate (*e.g., Fomes officinalis*). It is possible that this accelerated growth is caused by some nutritive portion of the extract, but such an explanation would hardly account for the subsequent slowing down (towards the normal growth rate) because, if this explanation were correct, the increased rate of growth would presumably be maintained as long as there remained an excess of medium to furnish nutrients. It is more likely that the phenomenon represents an initial response, followed by progressive desensitization to subinhibitory concentrations of a toxic constituent of the extract. The term *hormesis* (adj. *hormetic*) is proposed to designate such a stimulatory effect of subinhibitory concentrations of any toxic substance on any organism.

Acquired Resistance

Table 1 also shows that fungistasis decreased with time (*e.g., Fomes officinalis*) as if the fungi, after continued association with the toxic material, became able to tolerate or resist it, with a consequent increase in growth rate. This decrease in fungistasis suggested that a fungus might become adapted to high concentrations of toxic extract if it were gradually subjected to successively higher concentrations. In order to test this possibility plantings from the highest percentage of extract (in malt-agar medium) on which each fungus originally grew were transferred to a

TABLE 2.—*Concentrations of extract allowing growth before and after adaptation*

Species tested	Original tests		After adaptation
	Highest concentration on which growth occurred	Lowest lethal concentration	Highest concentration tested on which growth occurred
	Percentage concentration of extract in medium		
Trametes serialis	18	22–24[a]	24
Fomes roseus	14	22–24	24
Trametes subrosea	14	16–24	18
"Madison 517"	14	22–24	24
Lentinus lepideus	12	22–24	22
Polyporus sulphureus	12	14	24
Polyporus schweinitzii	6	14	14
Fomes pinicola	6	8	18
Coniophora puteana	4	6	14
Poria xantha f. *crassa*	4	6	14

[a] The precise lethal concentrations were not determined for the first five species.

[5] "Toxic" is used in the sense of injurious, whether fatal or not; "lethal," in the sense of fatal; and "fungistatic," in the sense of inhibitory but not lethal.

slightly higher percentage of extract. It was found that each of the fungi had become able to grow on these higher concentrations. The fungi were then re-transferred to still higher concentrations of extract. By this procedure it was found that each of the tested fungi could be made to grow on concentrations of extract well above the highest concentration on which each originally grew.[6] The pertinent data from these tests are recorded in table 2. The fungi undoubtedly could have been made to grow on much higher concentrations of the toxic extract by this method of adaptation.

Zone of Decoloration on Test Media

In media containing extract a zone of decoloration was observed extending a centimeter or less beyond the periphery of the mycelium of *Fomes officinalis*, *Fomes pinicola*, *Poria xantha* f. *crassa*, and *Trametes serialis* (Fig. 1, A, B). In plantings of *Poria xantha* f. *crassa* the zone of decoloration was present even around killed planting pieces (Fig. 1, C).

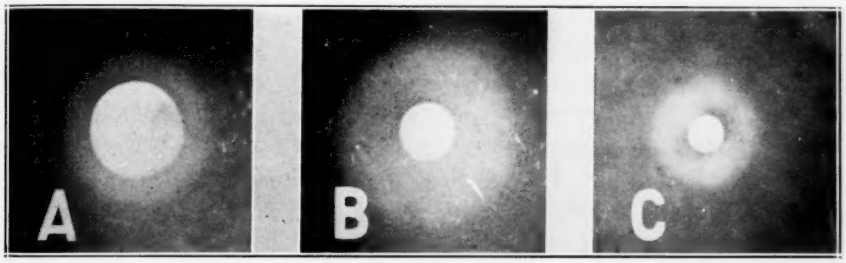

FIG. 1. Zone of decoloration in medium containing western red-cedar heartwood extract. A, decoloration around growing culture of *Fomes pinicola;* B, decoloration around growing culture of *Poria xantha* f. *crassa;* C, decoloration around killed planting piece of *P. xantha* f. *crassa.*

The possibility that this loss of color was the result of diffusion from the extract medium toward the planting pieces seems untenable for several reasons. Decoloration did not occur with all fungi, and was observed with killed planting pieces of only one fungus. Observations indicate that the color is held in an adsorption complex on the agar,[7] in which state diffusion would be impossible. The margin of the decolorized zone was quite definite, whereas if the loss of color were the result of simple diffusion into the planting piece a gradual transition would be expected.

The zone of decoloration, therefore, is considered to have resulted from leaching of metabolic products from the fungus into the surrounding

[6] *Fomes officinalis* was not included in these tests.

[7] Two facts indicate that the brown color of the extract is tied up in an adsorption complex when mixed with the medium: no Liesegang rings were present in the decolorized zone, which might be expected if the chromophore were capable of diffusion; the zone of decoloration was permanent, still being evident around the killed plantings of *Poria xantha* f. *crassa* two months after planting, whereas if diffusion of color were possible the decolorized zone would eventually darken. Apparently the chromophore is not colloidal when in water solution, because after filtering the extract through a Berkefeld filter the filtrate gave no indication of colloidality but still retained its brown color. It seems likely, therefore, that the color is held by adsorption to the particles of agar when in the medium.

medium where a chemical reaction produced the lighter color. The killed planting pieces of *Poria xantha* f. *crassa* must have produced enough of these metabolic substances before death to cause decoloration. Since this phenomenon occurred on media containing extract, and not on the control medium, it must have been the extract, and not the malt, that lost color.

Little is known concerning the chemistry of the cedar extract. Anderson and Sherrard (1) obtained two fractions from the aqueous extract, one of which they purified and identified as dehydroperillic acid. This substance was colorless and non-toxic to "Madison 517" in culture. They found the other fraction colorless also, toxic to "Madison 517," and probably phenolic.[8] Since phenolic compounds, although commonly colorless, characteristically oxidize to give a brownish color, it would seem to follow that the toxic portion of the extract becomes brownish on exposure to air.[9] If this be so, it is highly probable that the toxic portion of the extract is involved in the decoloration reaction. This would mean that the fungi causing the zone of decoloration do not grow in the presence of the toxic portion of the extract, but change it chemically (apparently to a non-toxic material) before advancing. This theory is strictly applicable only to those fungi in which the zone of decoloration was observed. There is no evidence at the present time to indicate the possible mechanism of growth of other fungi when subjected to cedar extract; yet it is quite possible that a similar mechanism holds for other fungi, the products of the reaction between the extract and the fungal product not being distinguishable, on the basis of color, from the unchanged extract.

Ability to overcome increasingly high concentrations of the toxic heartwood extract by virtue of this mechanism may well explain the advance of decay-producing fungi through the heartwood of living western red-cedar trees.

Other Cultural Characteristics on Extract Media

In young cultures (2–3 cm. in diameter) of "Madison 517" the extract medium beneath the planting piece was darkened to an orange-brown. This color advanced as the mycelium grew, the area beneath the planting piece fading to a straw color. On old cultures, which completely filled the plates, the orange-brown color eventually disappeared completely, leaving the medium straw-colored throughout. Since these color changes were more pronounced on higher concentrations of extract, and since the ring of

[8] The present study has also furnished evidence that the extract contains a phenolic group: (a) a very dark orange color is produced when a few drops of ferric chloride are added to the extract; (b) a white precipitate is formed with bromine water; (c) the extract, which is straw-colored when fresh, gradually darkers to dark brown upon exposure to air. (Growth tests with old, darkened extract gave the same results as tests with corresponding concentrations of fresh, light-colored extract, indicating that oxidation does not alter degree of toxicity, a characteristic that also holds for phenol.) Reactions a and b also occur after all colloidal particles have been removed by filtration through a Berkefeld filter.

[9] Possibly some non-toxic portion of the extract is also oxidized by atmospheric oxygen to give a brown color, in which case the decoloration might not concern the toxic portion of the extract exclusively.

orange-brown advanced as the mycelium grew, it appears that the color resulted from changes in the extract caused by the fungus. This is another indication that this fungus causes chemical changes in the extract, and does not merely grow in its presence, although there is no evidence to indicate whether this color is a product of the toxic portion of the extract or of some non-toxic part.

Certain of the fungi (notably *Polyporus sulphureus*), which produce azonate mycelium on ordinary malt-agar medium, exhibited pronounced zonation on media containing high concentrations of extract.

Mycelial mats were almost invariably thicker on media containing extract than on ordinary medium.

Incidental Data

When samples of the extract were allowed to evaporate to dryness, and weighed, 4.5 g. (average of 3 determinations) of extractives were obtained from each 100 g. of air-dry wood. This figure is probably low due to volatilization of solids during evaporation, because blocks (from another tree) when leached lost an average of 8.2 g. per 100 g. of oven-dry wood. Using this latter method Cartwright (2) obtained values of 5 per cent to 6.9 per cent.

The acidity of increasing concentrations of the extract was determined potentiometrically. It was found that the pH decreased rapidly to 3.5 for 8 per cent extract, beyond which there was very little change, a 24 per cent solution having a pH of 3.2. Such a curve is characteristic of the weak organic acids. Cartwright (2) reports that extracts from 2 trees showed pH values of 2.2 and 4.5. An attempt was made, with various cultures, to detect differences in pH between the decolorized zone and the unchanged medium beyond the decolorized zone. The results were so inconsistent that no conclusions could be reached.

High concentrations of extract prevented the agar from gelling completely, possibly due to its acidic reaction.

SUMMARY AND CONCLUSIONS

The hot-water-soluble extractives of western red-cedar heartwood were removed at 100° C. from sawdust and mixed, in various concentrations, with malt-agar medium. Several species of wood-decaying fungi were planted on these media and their growth rates were measured. Growth characteristics of the fungi in the presence of the toxic extract were recorded. The following conclusions were reached.

Extreme dilutions of the extract are hormetic (stimulatory), at least to some fungi.

The degree of hormesis decreases with the age of the culture.

Any considerable concentration of the extract is fungistatic, and in sufficient concentration the extract is lethal to the tested fungi.

The degree of fungistasis decreases with the age of the culture.

Fungi can be adapted to normally lethal concentrations of the extract if they are grown on successively higher concentrations of the extract in a malt-agar medium.

Some, at least, of the tested fungi secrete metabolic products that decolorize the surrounding medium and apparently overcome its toxicity.

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PHYTOPATHOLOGICAL NOTES

The Bulb or Stem Nematode on Alfalfa, Sweet Clover, and White Clover.—The bulb or stem nematode, *Ditylenchus dipsaci* (Kühn) Filipjev, has a host range comprising over 300 plant species. Within this nematode species there exist biologic strains that infect only one host, and others that infect several hosts. The experiments recorded here show that a strain of *D. dipsaci* from alfalfa was capable of infecting 3 different hosts.

In April, 1942, a field of alfalfa, *Medicago sativa* L., near Reno, Nevada, was observed in which a high percentage of the plants was infected with *D.*

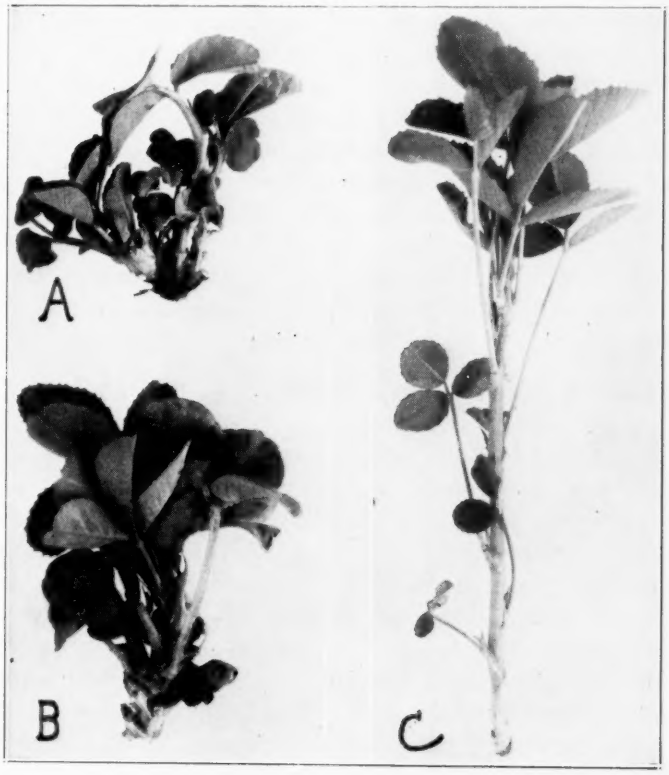

FIG. 1. Shoots of sweet clover plants. A and B. Naturally infected with *Ditylenchus dipsaci*. C. Not infected.

dipsaci. Many sweet clover plants, *Melilotus alba* Desr., were present in this field and at least 50 per cent bore swollen, distorted buds, heavily infected with stem nematode (Fig. 1). A few plants of white clover, *Trifolium repens* L., also were found with characteristic symptoms. Plants of red clover, *T. pratense* L., were present but apparently were not infected.

Under greenhouse conditions, cross inoculations were made between infected and noninfected alfalfa, sweet clover, and white-clover plants to de-

termine if the same strain of eelworm was parasitizing the 3 plant species found naturally infected in the field. Inoculations were made by scattering pieces of nematode-infected buds among the crowns of nematode-free plants and covering them with a thin layer of sand, kept moist to favor the nematodes. The following transfers were successful: alfalfa to alfalfa, alfalfa to sweet clover, sweet clover to alfalfa, sweet clover to sweet clover, and white clover to alfalfa. Check plants remained free from infection. Transfers were regarded as positive only when microscopic examination showed that the life cycle of the nematode was completed within the tissues of the host plant.

The experimental transfers, the natural plant infections in the field, and the fact that no apparent morphological differences exist among specimens from the three hosts, show quite definitely that this strain of *Ditylenchus dipsaci* was parasitic on alfalfa, sweet clover, and white clover.

Experiments under identical conditions, using alfalfa buds infected with *Ditylenchus dipsaci,* collected near Minden, Nevada, failed to produce infection on sweet clover. This may indicate that populations differing in host preference may exist in the two localities.—OLIVER F. SMITH, Associate Pathologist, Division of Forage Crops and Diseases, Bureau of Plant Industry, Agricultural Research Administration, U. S. Dept. of Agriculture, Reno, Nevada, and MERLIN W. ALLEN, Assistant Nematologist, Division of Nematology, Bureau of Plant Industry, U. S. Dept. of Agriculture, Salt Lake City, Utah.

Phoma terrestris in the Roots of Mature Maize Plants.[1]—In the autumns of 1941 and 1942, *Phoma terrestris* Hansen was isolated alone, and in combination with *Fusarium moniliforme, Pythium* sp., or miscellaneous fungi, from pink roots of plants of yellow dent corn grown on the University farm, Madison, Wisconsin.

Pycnospores were formed in culture in 1941; in 1941 and 1942 the rose-color hyphae and olive-brown "pycnidial primordia" characteristic of *Phoma terrestris*[2] were visible in the root tissues. The slender pink hyphae had penetrated the stele (Fig. 1, A); the pycnidial primordia were found most often in the cortical layers of the root, within the cells of the endodermis (Fig. 1, B), and in the small rootlets (Fig. 1, C). This organism differed from the common run of fungi in that the rose color of the hyphae remained unchanged during the process of killing, dehydration, and embedding. Formol-acetic alcohol was the killing solution used, followed by alcohol, chloroform, and paraffin.

The fungus was isolated from the plants of several single crosses, among which there appeared to be some differences in susceptibility to the root injury.

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Agricultural Research Administration, U. S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station.

[2] Hansen, H. N. Etiology of the pink-rot disease of onions. Phytopath. **19**: 691–704. 1929.

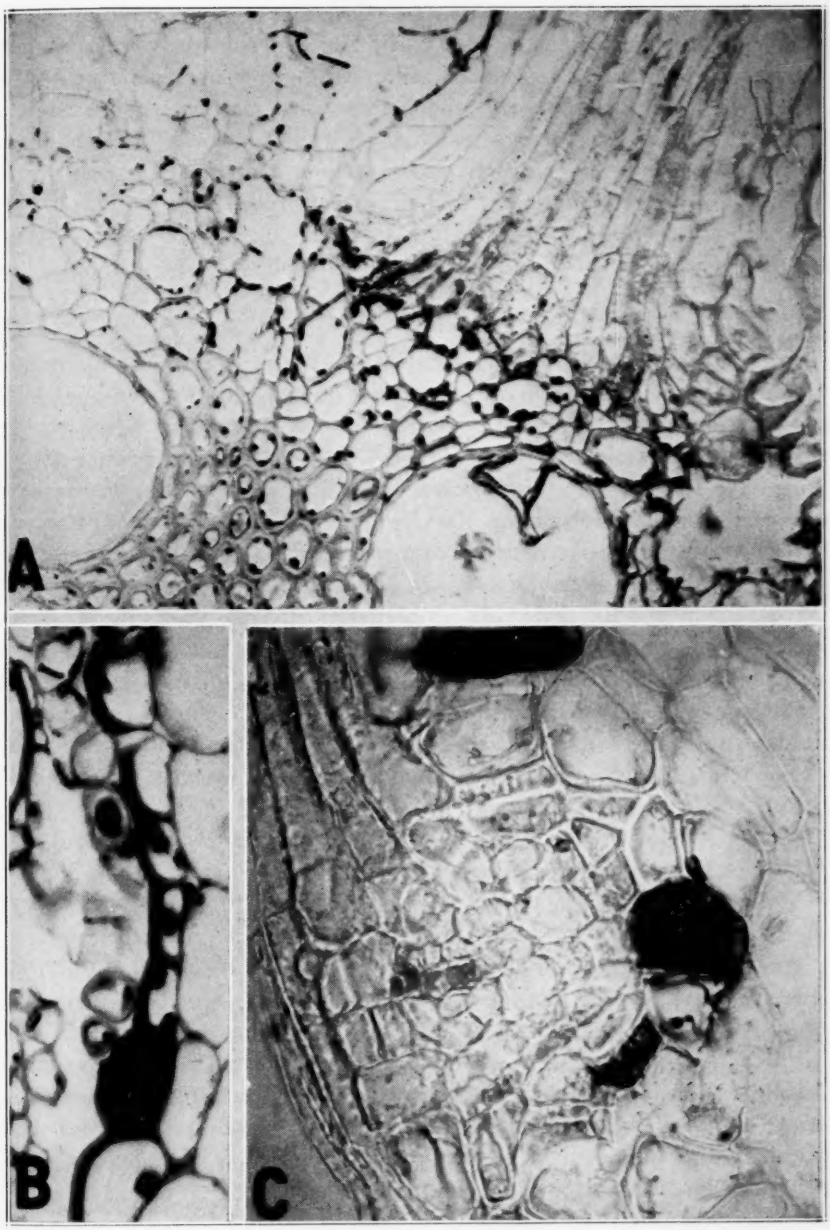

FIG. 1. Photomicrographs of paraffin sections of corn roots and rootlets showing *Phoma terrestris* within the tissues. A. Cross section of root showing the rose-color hyphae of the fungus in the cells and vessels of the stele. ×290. B. Cross section of root containing pycnidial primordium and olive-brown hyphae in the endodermal cells and rose-color hyphae in the stele. ×500. C. Pycnidial primordium in the cortex of a root, and both primordia and pink hyphae in the rootlet. ×425. All sections were lightly stained with fast green to render the host tissue visible. Safranin was used also in B, to differentiate the endodermal cells. The fungus needed no stain. (Photomicrographs made by Eugene H. Herrling, Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin.)

Kreutzer[3] included corn in his list of young crop plants slightly susceptible to attack by *Phoma terrestris*. His experiments were conducted with seedlings in flats of autoclaved soil. In the present case natural infection occurred in the field, and the plants were mature when examined. Consequently, it is not known at what stage in the development of the plants the fungus had entered the roots, nor the amount of injury caused by the attack. It is believed, however, that pink root of mature corn plants heretofore has not been ascribed to *P. terrestris* within the root tissue.—HELEN JOHANN, Department of Plant Pathology, University of Wisconsin, Madison, Wis.

Scolecospores in Diplodia macrospora.[1]—While examining platings of rotted corn kernels from a market sample of the 1942 crop, from Wye Mills, Maryland, a culture of *Diplodia macrospora* Earle with abundant scolecospores recently was observed. This is of two-fold interest in that scolecospores have been hitherto unreported in *D. macrospora*, and, according to the records in the Division of Mycology and Disease Survey, Bureau of Plant Industry, U. S. Department of Agriculture, this is the first recorded incidence of this species of *Diplodia* in Maryland.

Whether or not the scolecospores occurred separately or in conjunction with the pycnospores within the same pycnidium was not ascertained, inasmuch as both spore forms were seen only after a mount had been made of a small cluster of crushed pycnidia scraped from the surface of the corn kernel. In her discovery of scolecospores in *D. zeae*, in 1939, Miss Johann[2] described this spore form as occurring either alone or with the bicellular pycnospores in the pycnidium.

The relative infrequency of *Diplodia macrospora* in Maryland is attested by the fact that during the course of a corn-ear-rot survey, now going into the 10th consecutive year, approximately 3,000 cultures of *D. zeae* were plated from this State before the first culture of *D. macrospora* was found. —PAUL E. HOPPE, Division of Cereal Crops and Diseases, Bureau of Plant Industry, University of Wisconsin, Madison, Wis.

Plot Technique for Disease-control Studies on Fine Turf.—Investigation of chemical control of fine-turf diseases is conditioned by two major difficulties: 1. Paucity of experimental plots exhibiting the disease, and 2. Difficulty of finding sufficient area to minimize the error introduced by apportioning the small quantity of necessary chemicals.

Certain diseases, *e.g.*, dollar spot, are easily induced by artificial inoculation. The pathogen can be cultured readily on sterilized grain. If the

[3] Kreutzer, W. A. Host-parasite relationships in pink root of *Allium cepa*. II. The action of *Phoma terrestris* on *Allium cepa* and other hosts. Phytopath. 31: 907–915. 1941.

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Agricultural Research Administration, U. S. Department of Agriculture and the Wisconsin Agricultural Experiment Station.

[2] Johann, Helen. Scolecospores in *Diplodia zeae*. Phytopath. 29: 67–71. 1939.

inoculum be broadcast over the area, infection occurs in 2 to 3 days. Infection can be intensified by leaving the clippings of the first and second mowings after inoculation. Snowmold (*Typhula*) inoculum, sown broadcast, has given excellent results in 2 out of 3 years; yet, repeated attempts to induce large brown patch, Helminthosporium or Colletotrichum "melting-out" have failed. It has been necessary, therefore, to seek experimental areas on golf courses where these diseases occur naturally.

Golf-course greens vary from 3,500 to 7,000 square feet of turf. Generally of irregular shape, they present terrain of various levels and slopes. Many replications are needed to reduce the error of heterogeneity due to elevation, exposure, playing wear, and drainage. Areas of 1,000 square feet are de-

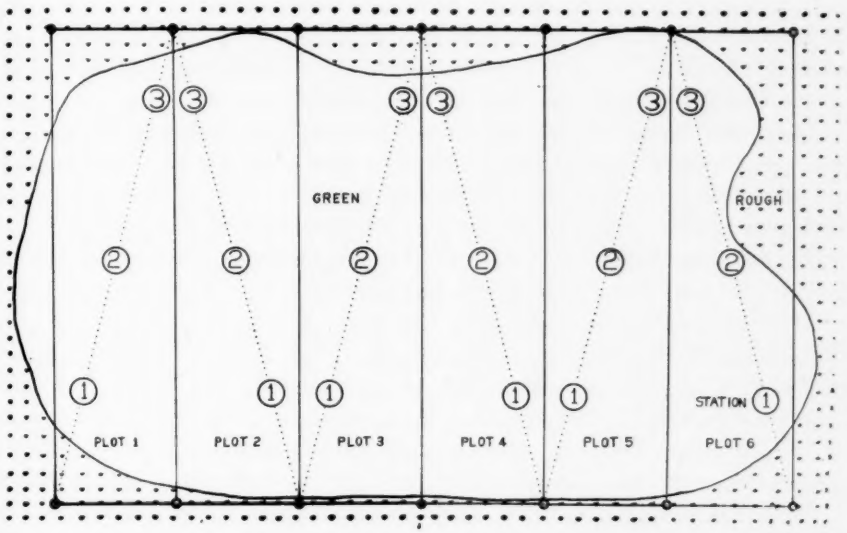

FIG. 1. Plot layout of 18th Green, West Course, Merion Golf Club, Ardmore, Penna.

sirable for treatment, since practical control measures are based on rates per 1,000 square feet. It would be almost impossible to lay out such plots on the average golf green without the aid of a surveyor. Moreover, treatments must be made intermittently throughout the growing season, a fact necessitating rapid relocation of the plots. These handicaps are easily overcome by the following technique:

Knowing the number of treatments to be used, estimate the area to be plotted, *e.g.*, 4 treatments—4,000 square feet; 6 treatments—6,000 square feet. Then measure long axis of green avoiding diagonals. Using measure of this long axis, calculate convenient width for rectangle required to give experimental area. With raised or cut-in greens, adjust width in direction of more accessible terrain. Locate corner stakes in rough (Fig. 1), drive to ground level to avoid interference with mower. Treat individual plot stakes in like manner. Whitewash each stake and small area surrounding it for subsequent easy relocation. Whitewash must be renewed once or twice because of growth and subsequent clipping of grass. On large and irregular greens residue areas often are big enough for replicate or additional treatments. On application of fungicide insert 6-inch spike behind each stake; stretch heavy twine to outline individual plots. Apply treatment uniformly over whole plot, rough and green alike.

For dollar spot, data may be satisfactorily taken by arbitrarily locating specific stations on a diagonal of each plot. If a 100-foot tape line be used to locate the diagonal, stations may be located at specific points. If infection is uniform the location of stations is a simple chore, but if scattered, one must be careful to insure representative areas. Three stations are used, each containing the area enclosed by a home-made wire hoop 3 feet in diameter. The spots per station are counted and averaged. Increase or decrease in average number of spots correlated strikingly with visual evidence of fungicide effectiveness.

For large brown patch and the "melting-out" complex, the station method was not so useful. A rating system had to be established employing the number of patches per foot of diagonal. Correlated with temperature and rainfall, this system worked satisfactorily. Data for each treatment were recorded weekly.—F. BEN STRUBLE, Graduate Stipend Scholar, Department of Botany, The Pennsylvania State College.

A Method for Obtaining Single-spore Cultures of Agaricus campestris.—To obtain single-spore cultures of *Agaricus campestris* it has heretofore been necessary to seed a mass of spores on a nutrient medium, and then isolate individual spores after they have germinated.[1,2] Although a high percentage of spores from such spore prints will often germinate, few single spores isolated from the same print will do so.[1] A method for germinating single spores would be useful because spores could be isolated with a micromanipulator and thus allow observation of germination and subsequent growth. Isolation and germination of pairs of spores from the same basidium would further facilitate genetic studies on this fungus.

It has been known for some time that spores of *Agaricus campestris* germinated best when in groups or in close proximity to growing mycelium of their species.[3] The assumption has been that a stimulatory substance may diffuse from the few germinating spores to others. These germinate and in turn stimulate others near them to germinate.

The writer attempted to use this stimulation in developing a method of inducing germination. A successful method is herewith described. A large number of uncontaminated spores were smeared on an agar slant with a sterile brush and allowed to germinate and grow for one to two weeks. After a solid mycelial mat had formed, the culture was killed by immersing the tube in boiling water until the medium was completely liquid. The dead mycelial mat was then removed aseptically and the sterile medium used for hanging drops on cover slips over Van Tieghem cells. From 30 to 50 per cent of the single spores of two commercial varieties of mushroom isolated and placed on this medium have germinated in replicated tests. Apparently the rate of germination is not so high for all varieties as one variety that resisted germination by mass seeding also germinated very poorly when single spores were isolated. A limited number of tests were made with medium staled by mycelial growth. A lower rate of germination was obtained here ranging between 20 and 30 per cent.

[1] Lambert, E. B. Principles and problems of mushroom culture. Bot. Rev. 4: 397–426. 1938.

[2] Lambert, E. B. A spore isolator combining some of the advantages of the LaRue and Keitt methods. Phytopath. 29: 212–214. 1939.

[3] Ferguson, M. A preliminary study of the germination of the spores of *Agaricus campestris* and other basidiomycetes. U. S. Dept. Agr., Bur. Pl. Ind. Bull. 16. 1902.

As checks against the experiments just described, 100 single spores were isolated and placed on unstaled medium and only about 5 per cent germinated. It would be difficult to obtain an adequate number of cultures in this way because of the large amount of work required. The new method should prove much more useful, as it insures a higher percentage of germination and thus makes it possible to get a more representative sample of the spore population with economy of time and effort.—D. J. DEZEEUW, Butler County Mushroom Farm Inc., West Winfield, Pa.

Host-parasite Relationship in Rust-infected Oats.—Initial cytochemical studies on the host-parasite relationship as presented by different varieties of oats (*Avena sativa*) infected by crown rust (*Puccinia coronata* Corda) were undertaken by the writers in 1942.

The varieties Bond, Bond × D69, Markton, Rainbow, Richland, and Victoria were inoculated with physiologic race 1 of *Puccinia coronata*. As soon as the rust sori had developed sufficiently for sectioning, freehand tangential sections of infected living leaf tissue were obtained from immediately beneath the sori and immersed in saline and sucrose solutions containing 1 per cent neutral red and stained *in vivo*. Like sections were immersed in a saturated solution of 2–6 dichloroquinone imide in a 2 per cent solution of sodium barbiturate buffered at pH 8.6. Still other sections were immersed in solutions of para-phenylenediamine, and molybdenum reagent, respectively.

Use of the above techniques afforded opportunity to note the comparative behavior of the phenolic compounds (pyridoxin, catechol, etc.), the polyphenol oxidases, and the distribution of the phosphorus compounds peculiar to the "resistant" or "susceptible" varieties of the host.

Response of the rust-infected host-plant cell may vary from sudden death of such cells to a progressive evolution of cell constituents making possible a prolonged survival of host cell and parasite. These extremes of response to infection, as well as all intermediate types of reaction, can now be interpreted cytochemically. A manuscript embodying the salient results of our investigation[1] of the problem is in preparation.—HARRY B. HUMPHREY and JEAN DUFRENOY, Bureau of Plant Industry Station, Beltsville, Md., and Louisiana State University, Baton Rouge, La.

[1] Grateful acknowledgment is here made to the members of the Department of Botany, Louisiana State University, for their collaboration and advice and for laboratory and greenhouse facilities placed at the disposal of the writers. The research here reported was conducted cooperatively by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture and the Louisiana State University.